A PHARMACEUTICAL COMPOSITION FOR TREATING RHEUMATISM AND THE PREPARATION THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

This application is a National Phase Patent Application and claims the priority of International Application Number PCT/CN2002/00246, filed on April 9, 2002.

THE FIELD OF THE INVENTION

The invention is ~~about~~directed to a medicine ~~which is used to treat~~for treating rheumatism, and ~~its~~the medicine's preparation.

THE BACKGROUND OF THE INVENTION

It is believed that ~~the~~ rheumatoid arthritis (RA) is refractory and about 18,000,000 RA patients have been disabled because of this disease. The ~~medicine reseach for~~medical research into curing RA has continued for about a century. Aspirin is the first medicine which ~~is~~was widely used to treat RA. The medicine to treat RA can be divided into 2 kinds: non-steroidal anti-inflammatory drugs (NSAIDs) and immunosuppressive ~~agent~~agents. NSAIDs includes cyclophthasine, antinfan and adrenal cortex hormone. ~~The clinical researchs have proved~~Clinical research has proven the effectiveness of NSAIDs. ~~The immuneosuppressive agent includes~~Immunosuppressive agents include methotrexate, cyclophosphane, penicillamine ~~and et al. The immunoregulation~~among others. Immunoregulation has become one of the important ~~theropies~~therapies in ~~the~~ recent years. But all the medicines ~~which are~~ used to treat rheumatism have serious side-

~~effect~~effects. ~~The~~A medicine ~~which~~that can treat rheumatism effectively and ~~non-poisonously hasn't~~ nontoxically has not been invented ~~by now~~before the present invention.

There are 3 directions in the research of ~~antirheumatic~~antirheumatics that should be emphasized. The first direction is NSAIDs and cytokine-~~antagon~~antagonists, such as ~~recombined~~recombinant, soluble ~~TNFαntagon~~TNFα antagonists, IL-1 ~~inhibitor~~inhibitors and PAF ~~inhibitor~~(platelet-activating factor) inhibitors. The second direction is the new immunosuppressive ~~agent~~agents and ~~immunomodulator~~immunomodulators, such as cyclosporin A. The third direction is the compound medicines.

In ~~the TCM~~traditional Chinese medicine (TCM), the research on ~~the "arthralgia disease"(equals~~"Bi Zheng" (equivalent to the definition of rheumatism or arthralgia in the modern medicine) can be traced back to the Han dynasty more than 1,500 years ago. Three prescriptions: "Ma Xing Shi Gan decoction", "Fangji ~~Hangqi~~Huangqi decoction" and "~~Wutong~~Wutou decoction"~~, which is~~ were used to treat "Bi Zheng" ~~were~~as recorded in the ~~medicine~~medical classics "Shanghan Lun" ~~wroted~~written by the famous doctor Zhang Zhongjing at that time. A wild plant called "huo ba hua" (Gelsemium ~~elegan s~~elegans Bentll ~~is a kind of wild plant in Sichuang~~) in Sichuan province ~~and it~~ has been ~~proved~~proven effective ~~to treat~~in the treatment of rheumatism ~~in a~~based on clinical research ~~carried at~~performed in the local area~~. But the further~~ (Sichuan province). However, subsequent study found that it had a serious side-effect on the ~~reproduction~~reproductive organs and ~~some~~ other uncontrollable ~~problem~~problems.

The treatment of "~~arthralgia disease~~Bi Zheng" by the ~~method~~methods of TCM has reached a high level after a long history of development by numerous doctors~~' development in so long a history. By now~~, Currently, there are many effective prescriptions and herbs. ~~There are more~~More than 80 kinds of herbs and 29 kinds of patent medicines are recorded in the China pharmacopoeia 1995 edition and 2000 edition. ~~But there are still~~However, many problems still remain: for example~~:~~, ① ~~the effect is not good enough~~ TCM is still ineffectual in treating ~~the~~ serious arthralgia ~~disease~~ such as rheumatoid arthritis; ② the dosage forms ~~are not fit for~~cannot meet the needs of modern life. ③ some medicine ~~has~~have good ~~effect~~effects, but the side-~~effect is serious~~effects are too damaging, such as when using the extract of *triperygium wilfordii*. ~~So that~~Thus, it is necessary to develop ~~the~~a new antirheumatic medicine that is highly-effective ~~lowly noxious~~, with minimal noxiousness, and convenient ~~for administration antirheumatic medicine~~to administer. This medicine should have ~~the~~ similar ~~effect~~effects and ~~the~~ lower side ~~effect to the~~-effects than artificial ~~antirheumatic~~, anti-rheumatic medicine.

~~THE CONTENT~~SUMMARY OF THE INVENTION

The invention ~~is to supply~~provides an antirheumatic~~, which~~ that is highly-effective ~~lowly noxious~~, has minimal noxiousness, and is convenient ~~for administration~~to administer, and its preparation thereof.

The ~~invented medicine's technical proposal is realized by using the~~medicine uses the following crude herbs ~~as following~~:

Tripterygium hypoglaucum (Levl.) Hutch~~.~~;

Epimedium brevicornum Maxim.~~,~~;

Lycium barbarum L.~~,~~; and,

Cuscuta chinensis Lam.~~,~~ (or *Cuscuta australis* R. Br.)

DETAILED DESCRIPTION OF EXEMPLARY EMBODIMENTS

The ~~invented medicine is made from the crude herbs above.~~antirheumatic medicine of the present invention utilizes the crude herbs as follows:

Tripterygium hypoglaucum (Levl.) Hutch;

Epimedium brevicornum Maxim;

Lycium barbarum L; and,

Cuscuta chinensis Lam (or *Cuscuta australis* R. Br.)

The ~~material~~crude herbs to produce the ~~invented~~antirheumatic medicine can be combined ~~on~~in several ways. The *tripterygium hypoglaucum* (Levl.) Hutch~~.~~ is the necessary herb~~,~~ with one or two or three of the other three herbs ~~can be~~ added ~~to made~~tomake the ~~material~~medicine.

One of the optimal proportional combinations of crude herbs ~~rate of~~for the ~~material~~medicine is as ~~following~~follows:

Tripterygium hypoglaucum (Levl.) Hutch. 1-4 ~~weightinweight~~parts by weight

Epimedium brevicornum Maxim. 1-4 ~~weightinweight~~ parts by weight

Lycium barbarum L. 1-4 ~~weightinweight~~ parts by weight

Cuscuta chinensis Lam. 1-4 ~~weightinweight~~ parts by weight

~~The other~~A second optimal proportional combination of crude herbs ~~rate of~~for the ~~material~~medicine is as ~~following~~follows:

Tripterygium hypoglaucum (Levl.) Hutch. ~~2 weightinweight~~ 2 parts by weight

~~Epimedium brevicornum Maxim. 2 weightinweight~~

~~Lycium barbarum L. 1 weightinweight~~

~~Cuscuta chinensis Lam. 1 weightinweight~~

Epimedium brevicornum Maxim. 2 parts by weight

Lycium barbarum L. 1 parts by weight

Cuscuta chinensis Lam. 1 parts by weight

The third optimal proportional combination of crude herbs ~~rate of~~for the ~~material~~medicine is as ~~following~~follows:

Tripterygium hypoglaucum (Levl.) Hutch. 1-4 ~~weightinweight~~parts by weight

Epimedium brevicornum Maxim. 1-4 ~~weightinweight~~parts by weight

The fourth optimal proportional combination of crude herbs ~~rate of~~for the ~~material~~medicine is as ~~following~~follows:

Tripterygium hypoglaucum (Levl.) Hutch. 2 ~~weightinweight~~parts by weight

Epimedium brevicornum Maxim. 2 ~~weightinweight~~parts by weight

The fifth optimal proportional combination of crude herbs ~~rate of~~for the ~~material~~medicine is as ~~following~~follows:

Tripterygium hypoglaucum (Levl.) Hutch 1-4 ~~weightinweight~~parts by weight

Epimedium brevicornum Maxim 1-4

~~weightinweight~~parts by weight

Lycium barbarum L 1-4

~~weightinweight~~parts by weight

The sixth optimal proportional combination of crude herbs ~~rate of~~for the ~~material~~medicine is as ~~following~~follows:

Tripterygium hypoglaucum (Levl.) Hutch 2

~~weightinweight~~parts by weight

Epimedium brevicornum Maxim 2

~~weightinweight~~parts by weight

Lycium barbarum L 1

~~weightinweight~~parts by weight

The seventh optimal proportional combination of crude herbs ~~rate of~~for the ~~material~~medicine is as ~~following~~follows:

Tripterygium hypoglaucum (Levl.) Hutch 1-4 ~~weightinweight~~ parts by weight

Epimedium brevicornum Maxim 1-4

~~weightinweight~~parts by weight

Cuscuta chinensis Lam 1-4

~~weightinweight~~parts by weight

The eighth optimal proportional combination of crude herbs ~~rate of~~for the ~~material~~medicine is as ~~following~~follows:

Tripterygium hypoglaucum (Levl.) Hutch 2

~~weightinweight~~parts by weight

Epimedium brevicornum Maxim 2 ~~weightinweight~~ parts by weight

Cuscuta chinensis Lam 1

~~weightinweight~~parts by weight

The content of the ~~icariine~~icariin ($C_{33}H_{40}O_{15}$) in the medicine ~~combinations~~ above ~~can~~should not be less than 2.0 mg.

The optimal proportional combinations of crude herbs ~~rate of~~for the ~~material~~medicine can be ~~the~~derived in other ~~way~~ways as ~~following~~follows:

Tripterygium hypoglaucum (Levl.) Hutch 1-4 ~~weightinweight~~parts by weight

Lycium barbarum L 1-4 ~~weightinweight~~parts by weight

And / or *Cuscuta chinensis* Lam ~~1-4~~ ~~weightinweight~~1-4 parts by weight

~~The optimal crude herbs rate of the material can be another way as following:~~

The optimal proportional combinations of crude herbs for the medicine can be derived in another way as follows:

Tripterygium hypoglaucum (Levl.) Hutch ~~2 weightinweight~~ 2 parts by weight

~~Lycium barbarum L 1 weightinweight~~

Lycium barbarum L 1 part by weight

And / or *Cuscuta chinensis* Lam 1 ~~weightinweight~~part by weight

The crude herbs are prepared ~~on the rate~~based on their proportional combinations and then they can be made into any dosage forms used in the clinic, such as the bolus form, the powder forms, the ointment forms, the tablet forms, the ~~sofe~~soft or hard capsule forms, the granule forms, the injection forms and so on.

The preparation method of the invented medicine is as

~~following~~follows:

The crude herbs are prepared based on the proportional weight~~-rate~~:

Tripterygium hypoglaucum (Levl.) Hutch 1-4

~~weightinweight~~parts by weight

Epimedium brevicornum Maxim 1-4

~~weightinweight~~parts by weight

Lycium barbarum L 1-4

~~weightinweight~~parts by weight

Cuscuta chinensis Lam 1-4

~~weightinweight~~parts by weight

The *Tripterygium hypoglaucum* (Levl.) Hutch. and *Epimedium brevicornum* Maxim are ~~smashed~~broken into pieces. Then the ~~powders~~pieces are decocted by water for 2~4 times separately. The *Lycium barbarum* L and *Cuscuta chinensis* Lam are soaked in the hot water (80~95℃) for 1~3 times separately. The ~~decocted~~decoction fluid and the immersion fluid of the herbs are collected and added separately to the ~~correspondent macroscopicvoid~~ corresponding column of adsorbent resins ~~column separately~~ having macroscopic voids. After ~~the~~ adsorption, the columns are washed with water until the flushing ~~liquor tunns~~liquid turns clear. Then the ~~columns~~resins are eluted ~~by~~with 60%-80% alcohol. The ~~eluting liquors~~eluted liquids are collected from ~~its~~the time when their color ~~turning~~turns deep ~~till the~~until their color ~~turning~~turns very weak. Then the remaining alcohol in the ~~upper part of the~~ column is pushed out by high pressure water and ~~mixed with the eluting liquor. The mixed eluting liquor is 3~8 times heavy of the correspondent~~is added to the eluted liquids. The combined eluted liquids

are about 3 ~ 8 times more concentrated than the corresponding crude herb in terms of the effective compounds. All ~~the 4 eluting liquors~~4 eluted liquids are ~~recycled and~~ condensed to ~~the~~a specific density ~~1.10 separately.~~of 1.10. The condensed liquors are ~~dehydrated~~dried by ~~spry~~a spray drying method to get the extract of the crude herbs. The 4 ~~kind~~kinds of extracts are mixed uniformly to be made into ~~any~~approproate dosage forms that are needed by the clinic.

The optimal preparation method of the invented medicine is as ~~following~~follows:

The crude herbs are prepared based on the proportional weight ~~rate:~~:

~~Tripterygium hypoglaucum (Levl.) Hutch 2 weightinweight~~

Tripterygium hypoglaucum (Levl.) Hutch 2 parts by weight

Epimedium brevicornum Maxim 2 ~~weightinweight~~ parts by weight

Lycium barbarum L 1 ~~weightinweight~~part by weight

Cuscuta chinensis Lam 1 ~~weightinweight~~part by weight

The *Tripterygium hypoglaucum* (Levl.) Hutch. ~~Is smashed~~is broken into pieces. Then the ~~powder is~~pieces are added with 13, 10, 10 ~~folds~~volume weight of ~~the~~ water to decoct ~~for~~ 3 times respectively. Each time is for 1 hour. The *Epimedium brevicornum* Maxim is cut ~~to piece~~into pieces. Then the ~~herb~~ pieces ~~is~~are added with 15, 10, 10 ~~folds~~volume weight of the water to decoct ~~for~~ 3 times respectively. Each time is for 1 hour. The *Lycium barbarum* L is ~~smashed to crude~~broken

into coarse powder and soaked in ~~the~~ hot water (80℃, 20 ~~fold~~volume weight of the crude herb) ~~for~~ 3 times. Each time is for 1 hour. The *Cuscuta chinensis* Lam is ~~smashed~~broken to ~~crude~~coarse powder and soaked in the hot water (80℃, 31 ~~fold~~volume weight of the crude herb) ~~for~~ 3 times. Each time is for 1 hour. The ~~decocted~~decoction fluid and the immersion fluid of the herbs are filtrated separately and added to the ~~correspondent macroscopicvoid~~corresponding column of adsorbent resins ~~column~~having macroscopic voids (the type of the resin is JD-1 (WLD resin)). After ~~the~~ adsorption, the resins in the columns are eluted ~~by~~with 70% alcohol. The ~~eluting liquors~~eluted liquids are collected from ~~its~~when the color ~~turning~~of the liquid turns deep ~~till~~until the color ~~turning~~of the liquid turns very weak. The alcohol is ~~recycled~~separated from the ~~eluting liquor~~eluted liquid. Then the ~~rest liquor~~ remaining eluted liquid is condensed and ~~dehydrated to get~~dried to obtain the extract powder. The 4 ~~kind~~kinds of extract powders are mixed uniformly to be made into any dosage forms that are needed by the clinic.

The invented medicine can be prepared ~~on~~by the method as ~~following~~follows:

The crude herbs are prepared based on the proportional weight ~~rate recorded before~~described above. The *Tripterygium hypoglaucum* (Levl.) Hutch. and *Epimedium brevicornum* Maxim are cut into pieces. The *Lycium barbarum* L and *Cuscuta chinensis* Lam are crushed or not crushed. The 4 ~~kind~~kinds of herbs are extracted ~~in the~~using 0~95% alcohol **at 10~98℃** for 1~4 times separately or together. The extracted ~~liquors~~liquids are mixed or not mixed. Then the extracted ~~liquors~~liquids are condensed, ~~dehydrated, smashed~~dried, broken into pieces and mixed

uniformly. The mixed powder can be made into any dosage form needed in the clinic.

The invented medicine can be made from the effective constituents of the 4 herbs.

The effective constituents of *Epimedium brevicornum* Maxim are ~~icariine~~icariin, icariside I , icariside II, and Icariin A. The effective constituents of *Tripterygium hypoglaucum* (Levl.) Hutch. ~~Are~~are diterpenes, triterpenes and alkaloids ~~compound~~compounds. The effective constituents of *Lycium barbarum* L and *Cuscuta chinensis* Lam are both ~~flavone~~flavones.

~~So that the~~The crude herb *Epimedium brevicornum* Maxim can be replaced by one or more kinds of the effective constituents of itself, such as ~~icariine~~icariin, icariside I , icariside II, and Icariin A. The crude herb *Tripterygium hypoglaucum* (Levl.) Hutch. ~~Can~~can be replaced by one or more kinds of the effective constituents of itself, such as diterpenes, triterpenes and alkaloids ~~compound~~compounds. While the *Lycium barbarum* L and *Cuscuta chinensis* Lam can be replaced by ~~flavone~~flavones.

It has been ~~proved~~proven by ~~the~~ pharmacodynamics research that the invented medicine (Fengshiping Capsule) ~~could~~can inhibit the primary and secondary injury adjuvant arthritis (AA). It ~~could~~can inhibit the delayed hypersensitivity (DTH) in the ear of ~~the~~a mouse caused by the 2,4 dinitrofluorobenzene (DNFB). It ~~could~~can inhibit the ~~antibody produce of the~~production of hemolysin antibody and the activity of the IL-1, IL-2, IL-6 and TNF in the macrophage and splenocyte. The Fengshiping Capsule ~~could~~can inhibit the lymphocyte transformation

induced by the ConA. It ~~could~~can inhibit the CD_4、CD_8 cells remarkably, especially CD_4 cells, but ~~the rate~~it does not affect the relative proportion of CD_4/CD_8 ~~was not affected~~ very much. There was a remarkable linear relationship between the dosage and the effect. Twelve to eighteen (12~18) g (crude medicine)/kg was the minimum effective dose. The invented medicine ~~could~~can inhibit the activity of the NK (Natural Killer) cells. ~~In~~At the effective dose, Fengshiping Capsule did not cause the atrophy of the important immune organs such as thymus and spleen, and did not inhibit the phagocytic activity of the macrophage.

The invented medicine had a remarkable ~~antiinfalmmatory~~anti-inflammatory action. It ~~could~~can inhibit the ~~over~~excessive penetrating ~~condition~~damage of the capillary in the mouse's abdominal cavity caused by the ~~ethanoic~~injection of acetic acid ~~injected~~. It ~~could improve~~can inhibit the swelling in the ear of the mouse caused by the croton oil. It ~~could~~can inhibit the pleuritis in the mouse and the assembling of the WBC (white blood cells) to the CMC cyst in the rat induced by the carrageenan. But the invented medicine ~~couldn't~~ cannot obviously inhibit the rat's foot swelling induced by the carrageenan and the granuloma caused by the tampon ~~obviously~~. The Fengshipng Capsule ~~could~~ can remarkably inhibit the body-twist reaction caused by the ~~ethanoic~~acetic acid in the mouse ~~remarkably~~.

Experimental example 1: the effect on the adjuvant arthritis (AA)

1.1 The ~~preventing~~preventive effect on the AA of the invented medicine

Seventy-two (72) isogenous SD rats of the same batch, half male and half female, 180 ~ 220g weight each, were divided randomly into 6 groups. Each group ~~has~~had 12 rats. ~~Every~~ Six (6) rats lived in a cage. The perimeter of the double ~~anklejoints~~ankle joints and the feet of the ~~rat~~rats were measured accurately and recorded as the normal value. All the rats were ~~drenched by~~given orally the same volume of the invented medicine ~~on the correspondent concentration or~~at different concentrations of the solution ~~of the Xihuangqi by the gastic injection.~~ with tragacanth. One (1) hour later, all the rats were injected with 0.1ml Freund's complete adjuvant (FCA) under the skin of the left ~~postpedes~~posterior limb. In the next 30 days, all the rats were ~~drenched with~~given orally the correspondent medicine once a day ~~on~~at the same dosage. And ~~in~~on these days, the ~~rats were measured of the~~ perimeters of the double ~~anklejoints~~ankle joints and the feet of the rats were measured once a day. In this experiment, the swelling degree (△cm)~~equaled to~~ equals the difference value of the perimeters measured after the FCA injection and before the FCA injection. (See the result in table 1.1 and 1.2) At the end of the experiment, the major organs of the rats were ~~weighted~~weighed. (See the table 1.3, 1.4)

Table 1.1 The effect of the Fengshiping on the swelling degree of the left ~~anklejoint~~ankle joint and foot after the injection of FCA in the rat AA model ($\overline{X} \pm S$)

Group	Dose (g/kg)	Swelling degree (△cm)						
		1d	2d	3d	9d	12d	14d	16d
Control	-	0.69±0.17	0.69±0.12	0.92±0.18	0.84±0.41	1.10±0.30	1.65±0.68	2.10±0.55
Fengshiping	7.5	0.74±0.12	0.66±0.074	0.83±0.13	0.77±0.27	1.11±0.45	1.34±0.53	1.91±0.61
Fengshiping	15	0.80±0.24	0.62±0.13	0.76±0.18	0.49±0.17*	0.73±0.34*	1.00±0.48*	1.38±0.67*
Fengshiping	30	0.75±0.19	0.67±0.19	0.87±0.28	0.63±0.22	0.73±0.34*	0.82±0.43**	1.05±0.53**
Tripterygium hypoglaucum (Levl.) Hutch.	5	0.72±0.11	0.68±0.16	0.91±0.18	0.66±0.23	0.88±0.29	1.03±0.36*	1.37±0.33*
prednisone	0.01	0.64±0.14	0.64±0.16	0.50±0.26	0.46±0.25	0.72±0.46*	0.87±0.46**	1.28±0.69*

Group	Dose (g/kg)	Swelling degree(△cm)					
		18d	20d	22d	24d	26d	28d
Control	-	2.18±0.44	2.05±0.46	2.00±0.46	2.04±0.57	1.92±0.65	1.83±0.67
Fengshiping	7.5	1.74±0.73	1.81±0.55	1.81±0.52	1.77±0.55	1.65±0.55	1.55±0.49
Fengshiping	15	1.32±0.59**	1.28±0.58**	1.34±0.61*	1.33±0.67*	1.20±0.64*	1.08±0.58**
Fengshiping	30	0.95±0.50**	0.87±0.51**	0.95±0.54**	0.89±0.59**	0.90±0.57**	0.86±0.51**
Tripterygium hypoglaucum (Levl.) Hutch.	5	1.47±0.43**	1.50±0.43**	1.49±0.43*	1.42±0.53*	1.40±0.56*	1.32±0.57
prednisone	0.01	1.18±0.7**6	1.03±0.67**	1.05±0.69*	0.90±0.64**	0.86±0.65**	0.85±0.59**

Comparing to the control group *P<0.05 , **P<0.01(the signs have the same meaning in the following tables)

1.2 The effect of the Fengshiping on the swelling degree of the left ~~anklejoint~~ankle joint and ~~footafter~~foot after the injection of FCA in the rat AA model ($\overline{X} \pm S$)

Group	Dose (g/kg)	Swelling degree (△cm)					
		2d	9d	12d	14d	16d	18d
Control	-	0.14±0.05	0.06±0.10	0.34±0.36	0.80±0.52	1.43±0.67	1.36±0.61
Fengshiping	7.5	0.18±0.06	0.10±0.14	0.26±0.36	0.82±0.52	1.31±0.64	1.28±0.71
Fengshiping	15	0.15±0.08	0.02±0.06	0.13±0.10*	0.37±0.31*	0.90±0.56*	0.79±0.60*
Fengshiping	30	0.18±0.09	0.06±0.06	0.16±0.08*	0.29±0.20**	0.49±0.41**	0.33±0.29**
Tripterygium hypoglaucum (Levl.) Hutch.	5	0.16±0.07	0.01±0.07	0.11±0.10	0.44±0.19**	0.87±0.56*	0.84±0.67*
prednisone	0.01	0.20±0.06	0.08±0.08	0.21±0.16	0.44±0.43	0.99±0.63	0.84±0.74*

Group	Dose (g/kg)	Swelling degree (△cm)				
		20d	22d	24d	26d	28d
Control	-	1.28±0.57	1.38±0.64	1.35±0.75	1.20±0.78	1.12±0.63
Fengshiping	7.5	1.33±0.71	1.31±0.73	1.27±0.73	1.16±0.73	1.07±0.65
Fengshiping	15	1.74±0.57*	1.92±0.61*	0.95±0.64*	0.88±0.58*	1.83±0.55
Fengshiping	30	0.27±0.30**	0.34±0.31**	0.32±0.33**	0.31±0.32**	0.34±0.32**
Tripterygium hypoglaucum (Levl.) Hutch.	5	0.82±0.65*	0.89±0.70*	0.80±0.67*	0.83±0.68	0.75±0.69
prednisone	0.01	0.82±0.72*	0.79±0.74*	0.75±0.67**	0.68±0.64*	0.71±0.67

1.3 The effect of the Fengshipng on the body weight of the AA rats ($\overline{X} \pm S$)

Group	Dose (g/kg)	Body weight change(g)		
		~~Initiative~~Initial BW	BW at 1 month later	BW change
Control	-	228±34	231±52	3
Fengshiping	7.5	229±34	220±46	-9
Fengshiping	15	223±40	232±34	9
Fengshiping	30	224±37	256±60	32
Tripterygium hypoglaucum (Levl.) Hutch.	5	226±45	230±43	4
prednisone	0.01	264±55	244±31	-21

1.4 The effect of the Fengshiping on the organ weight of the immune system in the AA rats (prevention experiment)($\overline{X} \pm S$)

Group	Dose (g/kg)	Organ index [(organ weight/body weight)/100]			
		Liver	Spleen	Thymus	adrenal gland
Control	-	3.92±0.65	0.34±0.10	0.098±0.040	0.027±0.01
Fengshiping	7.5	3.73±0.29	0.31±0.09	0.078±0.038	0.027±0.008
Fengshiping	15	3.48±0.32	0.38±0.10	0.100±0.034	0.023±0.005
Fengshiping	30	3.38±0.28*	0.44±0.12*	0.100±0.032	0.022±0.007
Tripterygium hypoglaucum (Levl.) Hutch.	5	3.21±0.30**	0.36±0.05	0.052±0.011**	0.026±0.009
prednisone	0.01	3.04±0.20**	0.32±0.08	0.050±0.060**	0.020±0.004*

1.2 The therapeutic effect on the AA of the invented medicine

Fifty (50) male SD rats were divided into 5 groups at random. The experimental model ~~building~~ was the same ~~to~~as the prevention experiment, but the correspondent medicines were ~~drenched~~given orally 13 days after the injection of the FCA. The medicines were ~~drenched~~given once a day for 2 weeks. The degree of swelling ~~degree~~ (△cm) was the difference of the perimeters between the value of first administration day and the other days. (~~Se~~See the result in table 1.5, 1.6) The major organs' weight is showed in table 1.7.

1.5 The therapeutic effect of Fengshiping on the ~~swelling~~ degree of ~~The~~swelling of the left anklejoint and foot in the AA rats ($\overline{X} \pm S$)

Group	Dose (g/kg)	Swelling degree (△cm)			
		1d	2d	4d	6d
Control	-	1.81±0.27	1.92±0.19	2.12±0.22	2.16±0.27
Fengshiping	7.5	1.68±0.50	1.64±0.54	1.70±0.57	1.82±0.61
Fengshiping	15	1.44±0.41*	1.51±0.36**	1.65±0.34**	1.74±0.31**
Fengshiping	30	1.50±0.56	1.48±0.41**	1.51±0.44**	1.59±0.51**
prednisone	0.01	1.78±0.51	1.70±0.51	1.63±0.50*	1.58±0.50**

Group	Dose (g/kg)	Swelling degree (△cm)			
		8d	10d	12d	14d
Control	-	1.92±0.32	1.87±0.34	1.92±0.39	1.78±0.44
Fengshiping	7.5	1.67±0.68	1.60±0.71	1.61±0.77	1.58±0.71
Fengshiping	15	1.46±0.37**	1.48±0.30*	1.28±0.37**	1.22±0.38**
Fengshiping	30	1.29±0.58**	1.29±0.65**	1.26±0.67**	1.20±0.68*
prednisone	0.01	1.27±0.46**	1.09±0.54**	0.94±0.50**	0.94±0.42**

1.6 The therapeutic effect of Fengshiping on the ~~swelling~~ degree of swelling of the right anklejoint and foot in the AA rats ($\overline{X} \pm S$)

Group	Dose (g/kg)	Swelling degree (△cm)			
		2d	4d	6d	8d
Control	-	0.36±0.26	0.45±0.25	0.55±0.34	0.47±0.29
Fengshiping	7.5	0.12±0.25	0.34±0.32	0.48±0.41	0.28±0.38
Fengshiping	15	0.21±0.18	0.38±0.27	0.44±0.33	0.21±0.33*
Fengshiping	30	0.10±0.48	0.06±0.28**	0.11±0.24**	0.06±0.27**
prednisone	0.01	0.10±0.13*	0.15±0.28*	0.11±0.25**	-0.08±0.34**

Group	Dose (g/kg)	Swelling degree(△cm)		
		10d	12d	14d
Control	-	0.48±0.25	0.46±0.31	0.40±0.36
Fengshiping	7.5	0.35±0.30	0.30±0.29	0.30±0.35
Fengshiping	15	0.19±0.45*	0.06±0.31**	-0.06±0.34**
Fengshiping	30	0.02±0.39**	0.05±0.38*	-0.02±0.41**
prednisone	0.01	-0.13±0.28**	-0.26±0.36**	-0.33±0.39**

n = 10 , comparing with the control group , *P<0.05 , **P<0.01

1.7 The effect of the Fengshiping on the organ weight of the immune system in the AA rats ($\overline{X} \pm S$)

Group	Dose (g/kg)	Organ index [(organ weight/body weight)/100]			
		Liver	Spleen	Thymus	adrenal gland
Control	-	0.35±0.23	0.35±0.061	0.073±0.014	0.026±0.0071
Fengshiping	7.5	3.21±0.52	0.33±0.091	0.071±0.026	0.024±0.0085
Fengshiping	15	3.40±0.54	0.36±0.014	0.067±0.022	0.023±0.0048
Fengshiping	30	2.79±0.43	0.32±0.083	0.069±0.029	0.023±0.0072
Tripterygium hypoglaucum (Levl.) Hutch.	5	3.92±0.59	0.35±0.100	0.075±0.034	0.027±0.0060
prednisone	0.01	3.52±0.35	0.28±0.047*	0.05±0.011**	0.02±0.0043*

The data ~~showed~~shown in the ~~table~~tables 1.1, 1.2, 1.3, 1.5 and 1.6 ~~proved~~prove that the Fengshiping ~~could~~can strongly inhibit the primary and secondary injury caused by FCA, whenever the medicine was ~~drenched~~given at the beginning of the FCA injection or 2 weeks after the FCA injection. The experiments ~~proved~~prove that the Fengshiping ~~had~~has both the ~~preventing~~preventive and the therapeutic effect. By comparing the effect of Fengshiping on the degree of swelling ~~degree in~~of the anklejoint and foot, we found that the Fengshiping ~~could~~can inhibit the specific ~~immunoswelling in the anklejoint~~immuno-swelling of the ankle joint better than the nonspecific ~~immunoswelling~~immuno-swelling in the foot of rats. This result ~~indicated~~indicates that the main effect of Fengshiping was inhibiting the ~~immunity~~ inflammatory reaction of the immune system.

The data in the ~~table~~tables 1.3, 1.4 and 1.7 ~~showed~~show that the AA rats had no obvious BW (body weight) increase during the period of the experiment. In the group ~~drenched of~~given the Fengshiping ~~on~~with the effective dosage, the rats ~~still had BW~~continued to increase in BW. In the groups ~~of~~treated with prednisone ~~and preventing~~, the BW of rats ~~had~~ decreased, while the thymus and adrenal gland ~~were~~ atrophied. In the

group ~~of~~treated with *tripterygium hypoglaucum* (Levl.) Hutch, the thymus had ~~thrinked yet. But in~~not atrophied. In the 3 groups ~~drenched with~~given the different dosage of Fengshiping, ~~on~~no atrophy of the thymus and adrenal gland were observed.

1.3 The pathologic change of the AA after the treatment of the invented medicine in rats

Forty-five (45) SD rats, 180±20g weight each, were divided into 6 groups. After the AA caused by FCA appeared, all the rats were ~~drenched with~~given orally, Fengshiping solution ~~by gastic injection~~ for 5 days once a day. One (1) hour after the last administration, the joint index of the rats was measured and calculated. The ~~secondary injuried postpedes'~~damaged joints ~~on~~of the ~~opposite of~~posterior limbs distal from the FCA ~~injection~~injection were ~~taken off~~removed and soaked in ~~the~~ formaldehyde. After the tissues were ~~HE tinted~~stained with HE (hematoxylin-eosin), the pathological change of the synovium and cartilage were observed and recorded. The data ~~were showed~~are shown in table 1.8.

1.8 The effect of Fengshiping on the AA joint index in the rats ($\overline{X} \pm S$)

Group	Dose (g/kg)	Rat number	Joint index
Control	-	8	0**
AA model	-	7	6.2±0.49
Fengshiping	7.5	9	4.86±0.90**
Fengshiping	15	7	4.71±0.95**
Fengshiping	30	7	4.56±1.13**
Glucosidorum Tripterygll Totorum	0.006	7	4.57±0.79**

Comparing with the model groupP<0.01**

The joint index was the sum of the inflammatory scores of the four limbs. According to the degree of inflammatory, each limb was evaluated on the criteria as following: normal (0), red without swelling (1), red and swelling (2), seriously swelling (3), ~~deforming~~deformity and ~~tetanus~~stiffness (4).

Observed from the microscope, the joint synovial membranes of the rat posterior limb were ~~hyperplasia~~hyperplastic in the ~~model~~control group; ~~and~~ the collagen fiber had increased; and there was infiltration of lymphocytes and plasma cells in the tissue. ~~The~~An obvious granuloma had formed. The synovial cells had degenerated and the cytochylema ~~had been~~was tinted red; the ~~caryon~~nucleus had ~~been pycnosis~~become pycnotic; the epithelium had exfoliated in some part of the synovial membrane. The cartilage ~~turned atrophy; the~~became atrophied; its surface ~~of it~~ was rough and some of the chondrocytes had proliferated ~~lightly~~slightly.

After the treatment ~~of~~with the Fengshiping, the inflammation of the joint synovial membrane was inhibited, more ~~collogen~~collagen fiber was produced; less synovial cells exfoliated ; the cells on the surface of the cartilage had proliferated and the surface had turned smooth. The cartilage was ~~on the~~in a recovering condition.

Experimental example 2: The effect of Fengshiping on the delayed typed hypersensitivity reaction (DTH) caused by 2,4-DNFB ~~in~~(dinitrofluorobenzene) administered to the ear of the mouse

Fifty (50) NIH mice, half male and half female, were divided into 5 groups. Each mouse was ~~led to~~induced into hypersensitivity ~~reaction by using the~~by application of 1% DNFB acetone solution ~~on the~~at a dosage

of 0.025ml at the right ~~place~~side of the abdomen where ~~the pilus~~fur had been ~~cut yet~~removed. Using the same solution ~~on~~at the same place ~~was to enhance~~enhanced the ~~hypersensitivity~~ reaction on the third day. On the fifth day, all the mice were smeared with the 1% DNFB edible oil solution at the mice's right ears ~~on the~~at a dosage of 0.01 ml each. Twenty-four (24) hours later, all the mice were killed. The mouse' s 2 ears were ~~weighted~~weighed by the torsion balance and the difference of the 2 ears was recorded as the ~~DTH~~ degree of DTH caused by the DNFB. The experiment was carried out ~~on the~~using different immune-inhibitory compounds and administration processes.

2.1 The effect on the DTH by the full course administration

The immune and administration processes is as ~~following~~follows:

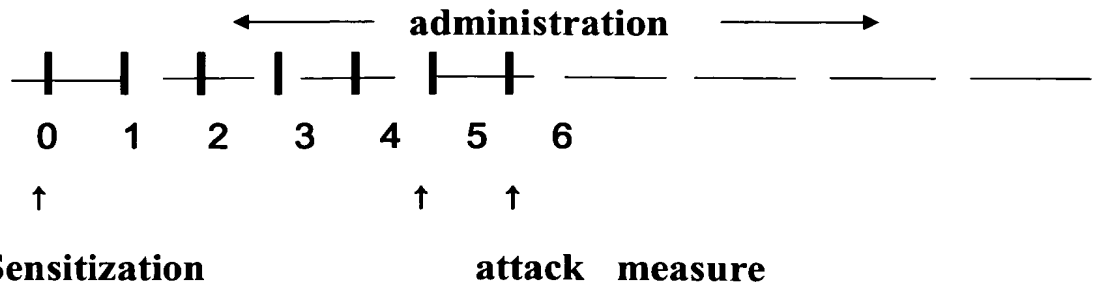

Table 2.1 The effect of Fengshiping on the DTH caused by DNFB in the NIH mouse ($\overline{X} \pm S$)

group	dose (g/kg)	Administration time (day)	Mice number	Percent of ear swelling	Percent of inhibition (%)	P value
control			10	34.20±3.77		
Fengshiping	27	0~5	10	26.24±3.34	23.3	<0.01
Fengshiping	40	0~5	10	12.99±4.96	62.0	<0.01
Fengshiping	60	0~5	10	10.43±7.53	69.5	<0.01
cortisumman	0.003	0~5	10	13.93±4.41	59.3	<0.01
control			10	42.43±5.28		
Fengshiping	40	-2~0	10	31.50±10.52	25.0	<0.01
Fengshiping	40	-2~2	10	30.88±7.92	27.2	<0.01
Fengshiping	40	-2~5	10	21.07±4.62*	50.3	<0.01
Fengshiping	40	5~6	10	32.00±9.37	41.7	<0.01
cyclophosphane	0.05	-2~2	10	39.40±10.78	8.1	>0.05
cyclophosphane	0.05	-2~0	10	37.47±6.71	11.7	>0.05
control			10	38.50±4.67		
cyclophosphane	0.1×3	0、2、4 day once a day	10	23.00±7.65	40.3	<0.01
cyclophosphane	0.25	-3d	10	41.84±7.75	-8.7	
Fengshiping	60	0~4	10	27.20±10.20	29.4	<0.01
cyclophosphane +Fengshiping	0.25 + 60	-3,0~4	10	38.07±6.65	1.1	

*comparing with the other groups P<0.05或P<0.01

~~According to the~~The data ~~showed~~shown in table ~~2.1, it indicated~~2.1 indicate that the Fengshiping had ~~a~~an obvious ~~inhibiting~~inhibitory effect on the DTH caused by DNFB. There was a significant relationship between the dosage and the effect. The ~~inhibiting~~inhibitory activity ~~increases~~increased when the dosage ~~adds~~increased. The ~~inhibiting percent~~inhibitory effect could reach 69.5% ~~on the~~at a dosage of 60.9g/kg.

2.2 The effect on the DTH ~~of~~with the different administration time

The corresponding results from using different immune-inhibitory compounds and administration processes ~~and the correspondent results had been showed in~~are shown in the middle and bottom parts of ~~the~~ table 2.1. According to the results ~~showed~~shown in the middle part of the table 2.1, all the different administration ~~ways could~~can significantly inhibit ~~the~~ DTH of the mouse ~~in spite of the administration beginning from the 2 days before the sensitization and ending at the sensitization, or beginning from the 2 days before the sensitization and ending~~regardless whether the administration began from 2 days before the sensitization to the end of sensitization, or whether it began from 2 days before the sensitization to 2 days after the end of sensitization, or ~~beginning from the 2 days before the sensitization and ending~~whether it began 2 days before the sensitization to 5 days after the end of sensitization, or ~~beginning~~whether it began before the attack and ~~ending~~end after the attack. ~~But~~However, the administration ~~way~~ that began 2 days before the sensitization and ended 5 days after the sensitization had the most powerful ~~inhibiting~~inhibitory activity. ~~It indicated~~The data indicate that the Fengshiping could inhibit the DTH by ~~a compound mechanism that~~

it couldmultiple mechanisms: it can inhibit the cells participanttparticipating in the early period of the DTH, and it can inhibit the effector cells in the advancedlatter period and theas well as cells related to the DTH in the middle period. This mechanism of inhibition by Fengshiping was different from that of the cyclophosphane. OnUsing a small dosage, the cyclophosphane didn'tdid not affect the DTH, if its administration way was beginning from thebegan 2 days before the sensitization and endingended at the day of sensitization day or 2 days after the day of sensitization day.

Based on the bottom part of the table 2.1, if a high dosage of cyclophosphane was drenchedgiven to the mouse inat one time 3 days before the sensitization, the function of the Th (T helper) cells would turnbecome sthenic because of the powerful inhibition on the Ts (suppressor T) cells. The DTH in the mouse would be enhanced. If the cyclophosphane was used with the Fengshiping oninin this administration waymethod, it could lower the inhibitinginhibitory activity of Fengshiping. This result indicatedindicates that the Fengshiping havehas a different machnizmmachnism compared to the cyclophosphane in the control of DTH. The Fengshiping maybe hadmay have a higher activity in inhibiting the THcellsTh cells.

Experimental example 3: The effect on the humoral immunity

3.1 The effect on the producelevels of the hemolysin antibody causedinitiated by the chick RBCCRBC (chicken red blood cells)

One hundred ninety (190) mice, 18-22g weight, half male and half female, were divided into 19 groups. Each mouse was immunized with

0.2 ml of 5% CRBC solution ~~0.2 ml. The.~~ Fengshiping solutions were ~~drenched~~given orally to the mice at the different times. Seven (7) days after the immunization, blood samples from all the mice were ~~sampled the blood~~taken from the eyes. Then the blood samples were diluted and the levels of the hemolysin antibody were measured ~~the level of the hemolysin antibody~~. The results ~~were showed~~are shown in table 3.1, 3.2 and 3.3.

Table 3.1 The effect of Fengshiping on the ~~produce~~levels of the hemolysin antibody in the NIH mouse ($\overline{X} \pm S$)

group		dose (g/kg)	Administration time	Mouse number	Hemolysin value	~~Inhibiting~~Inhibitory percent (%)	P value
control				10	169.0±62.0		
Fengshiping		18	0~7	10	46.0±15.6	72.8	<0.01
Fengshiping		27	0~7	10	35.4±12.0	79.1	<0.01
Fengshiping		40	0~7	10	28.2±5.9	83.3	<0.01
Fengshiping		60	0~7	10	16.7±3.0	90.1	<0.01
Tripterygium hypoglaucum Hutch.	(Levl.)	13.3	0~7	10	121.0±88.0 **	28.4	<0.015
cyclophosphane		0.02	0~7	10	35.0±12.0	79.3	<0.01

**** comparing with the Fengshiping (40g/kg) group P<0.01**

Table 3.2 The effect of Fengshiping on the ~~produce~~levels of the hemolysin antibody in the ICR mouse ($\overline{X} \pm S$)

group		dose (g/kg)	Administration time	Mouse number	Hemolysin value	~~Inhibiting~~Inhibitory percent (%)	P value
control		-	-	10	124.70±42.60		
Fengshiping		12	0~7	10	75.00±53.10	39.9	<0.05
Fengshiping		18	0~7	10	45.60±22.70	63.4	<0.01
Fengshiping		27	0~7	10	29.10±22.10	76.8	<0.01
Fengshiping		40	0~7	10	28.20±5.30	77.4	<0.01
Tripterygium hypoglaucum Hutch.	(Levl.)	6.0	0~7	10	143.50±67.90**		>0.05

cyclophosphane	0.02	0~7	10	27.80±6.60	77.9	<0.01

**comparing with the Fengshiping (18g/kg) group P<0.01

Table 3.3 The effect of Fengshiping on the ~~produce~~levels of the hemolysin antibody in the ICR mouse ($\overline{X} \pm S$)

group	dose (g/kg)	Administration time	Mouse number	Hemolysin value	~~Inhibiting~~Inhibitory percent (%)	P value
control	-	-	10	256.0±26.0		
Fengshiping	18	-7~7	10	198.0±50.0	22.7	<0.01
Fengshiping	18	-3~7	10	156.0±85.0	39.1	<0.01
Fengshiping	18	0~7	10	98.0±35.0	61.7	<0.01
cyclophosphane	0.02	0~7	10	25.0±4.0	90.2	<0.01

According to the data in table 3, ~~it indicated that the~~ Fengshiping ~~had~~has a remarkable ~~inhibiting~~inhibitory effect on the ~~produce~~levels of the hemolysin antibody in the different mouse species and this effect would increase along with ~~the~~an increase of the dosage. There was a certain relationship between the dosage and the effect. The lowest effective dosage was 12g/kg. ~~Comparing~~Compared with the same quantity of Tripterygium hypoglaucum (Levl.) Hutch, the Fengshiping had a higher ~~inhibiting~~inhibitory activity. Based on the data in table 3.1, the ~~inhibiting~~inhibitory activity of Fengshiping was 2.25 times higher than the Tripterygium hypoglaucum (Levl.) Hutch. The ~~inhibiting~~inhibitory activity of Tripterygium hypoglaucum (Levl.) Hutch. ~~On~~with the dosage of 13.5g/kg was weaker than that of the Fengshiping which ~~containing~~contains 6g/kg Tripterygium hypoglaucum (Levl.) Hutch).

3.2 The effect of the Fengshiping on the humoral immunity in the AA mouse

The NIH mice, 20±2g weight, were injected with 0.05 ml of FCA

under the ~~vola~~ skin of the right ~~postpedes.~~ posterior limb. Three (3) weeks ~~late~~later the AA model mouse ~~builded~~was established. The model mice were divided into 6 groups randomly and ~~drenched with the correspondent~~given orally the corresponding medicines for 5 days. At the beginning of the administration, all the mice were sensitized with 0.5ml of 10% sheep RBC (SRBC). Five days later, all the mice were killed. Their spleens were taken out and washed ~~by the~~with Hank's ~~liquor~~buffer to prepare the lymphocyte in suspended ~~liquor~~solution. The concentration of the cells was adjusted to 2×10^7 / ml. One (1) ml of lymphocyte suspension, 1 ml of 0.2% SRBC and 1 ml of 1:30 addiment were added to one test tube. The tube was put in the water bath at 37℃ for 1 hour. Then the tube was centrifugated at 2000rpm for 5 minutes. The supernatant fluid was separated and tested for its ~~sptical~~optical density at the 415nm wavelength on ~~the 722 apeotrophotometer~~a spectrophotometer. The value was ~~the represent~~representative of PFC (plaque-forming cell) quantity.

The other ~~share~~portion of the blood samples ~~got~~ from the sensitized mice was ~~separated~~used to isolate the serum to test the potency of the antibody. The measured data were recorded ~~on the way of~~using Log2 value. (See the data in table 3.4)

Table 3.4 The effect of Fengshiping on the humoral immunity in the mouse ($\overline{X}\pm S$)

group	dose (g/kg)	Mouse number	PFC (OD)	IgM(Log2)
control	-	8	0.819±0.013#	6.875±0.641
AA model group	-	10	0.940±0.019**	7.700±0.599*
fengshiping	5	8	0.834±0.012**#	6.875±0.641#

fengshiping	10	8	0.834±0.012**#	6.750±0.886#
fengshiping	20	8	0.830±0.014**#	6.375±0.518##
Glucosidorum Tripterygll Totorum	0.012	10	0.835±0.015**#	6.950±0.597#

Comparing with the control group *P<0.05, **P<0.01; comparing with the model group # P<0.05, ## P<0.01

According to the table 3.4, the levels of PFC and IgM in the AA mouse were higher than that of the normal mouse. ~~The~~ Fengshiping ~~could~~can lower the ~~produce~~levels of the PFC and IgM in the AA mouse significantly.

Experimental example 4: The effect of ~~the~~ Fengshiping on the passive ~~cutis~~cutaneous anaphylactic reaction (PCA) in the rat.

The rats were injected with the egg albumn at 10mg/kg in the muscle. At the same time, all the rats were injected with 2×10^{10}(0.2ml) bordetella pertussis in the abdominal cavity for sensitization. Two (2) weeks later, all the rats were killed to sample the blood. All the blood samples were separated ~~for preparing~~to obtain the serum.

Sixty (60) rats, 150~200g, half male and half female, were divided into 6 groups at random. In the light narcosis condition induced by ~~aether~~ether, each rat was ~~cut the fleece in the~~shaved on its back and injected with the 2x 0.1 ml concentrations of anti-egg-album serum ~~0.1ml~~ under the ~~skin at~~shaved area of the ~~fairless place~~skin. The serums were diluted ~~on the rates~~to concentrations of 1:5(d1) and 1:10(d2) before the experiment. Forty-eight (48) hours later, all the rats were ~~attacked by intravenous injecting the 0.5% evans~~intravenously injected with 0.5% Evans blue normal saline solution 1 ml which ~~containing~~contained 1 mg egg albumin. Twenty (20) minutes later, the rats were killed by decapitation. The skins on the rats' back ~~skin~~ were dissected and ~~turned over~~examined. According to the ~~darkandlight~~dark and ~~area~~light areas of

the blue stains exudated from the vessels, all the rats were evaluated by several people. The ~~skin~~skins stained by the ~~evens~~Evans blue were scissored and soaked in 5ml of 0.1% sodium sulfate acetone (7:3) solution for 48 hours. Then it was ~~centrifugated~~centrifuged to separate the supernatant ~~liquor~~liquid. The optical density of the supernates ~~were~~was measured ~~the optical density~~ at the wavelength 590nm to calculate the degree of the PCA reaction and the ~~inhibiting~~inhibitory percent. The results were shown in table 4.

Table 4 The effect of Fengshiping on the PCA in rat ($\bar{X} \pm S$)

Group	dose (g/kg)	value		absorbancy	
		d_1	d_2	d_1	d_2
Control	-	5.60±1.78	2.40±2.46	0.191±0.129	0.096±0.106
Fengshiping	12	7.50±2.51	4.20±2.49	0.402±0.213*	0.192±0.175
Fengshiping	24	7.10±2.13	4.10±1.79	0.310±0.177	0.137±0.099
Fengshiping	48	6.00±1.83	1.70±1.95	0.121±0.109	0.024±0.026*
Tripterygium hypoglaucum (Levl.) Hutch.	8	6.11±1.27	2.56±1.67	0.223±0.122	0.074±0.045
Ketotifen	0.1	2.78±1.64**	0.67±1.41	0.033±0.024**	0.027±0.019*

Comparing with the control group *P<0.05 , **P<0.01

~~According to the~~The data in table ~~4, it indicated~~4 indicate that the Fengshiping had a weak effect on the PCA in the rat. Only ~~on~~at a high dosage~~,~~ was the ~~inhibiting~~inhibitory effect of Fengshiping ~~was~~ obviously different from that of the control group.

Experimental example 5: The effect of Fengshiping on the cytokines.

5.1 The effect of Fengshiping on the levels of TNFα and IL-2 in the mouse.

Sixty (60) ICR mice, 18~22g, half male and half female, were divided into 6 groups at random. Each group was ~~drenched of~~given

orally the ~~correspondent~~corresponding medicines including the different dosages of Fengshiping and the other medicines. The medicines were administrated once a day for 10 days. Twenty-four (24) hours after the last administration, samples from the mice were ~~sampled~~taken, including the ~~macrophage~~macrophages and spleen cells from the abdominal cavity in the aseptic condition. The samples were washed with Hank's ~~liquor for 2 times~~buffer twice and with non-serum RPIM 1640 liquor ~~for 1 time~~once. Then the washed samples were diluted to ~~the~~a suspension with ~~the~~ 5% FCS-RPMI 1640 at ~~the~~a concentration of 2×10^8 cells / ml. Then the suspensions were added with 10ng/ml LPS (lipopolysaccharide) or the 10ng/ml ConA (concanavlin A) and cultured in the 5% CO2 condition for 48 hours at 37℃. Then the ~~cultured suspension were measured the~~ TNF α and IL-2 levels ~~on~~in the suspensions were measured using the usual methods.

The measurement of TNFα

The ~~batten~~plate was coated ~~by~~with mouse TNF-α monoclonal antibody. The ~~batten was added with the~~plate had cultured supernate ~~on~~added at the dose of 50μl/~~hole~~well. Then the ~~batten~~plate was ~~put still~~incubated for 60 minutes at ~~the~~ room temperature. Then the ~~batten~~plate was ~~added~~mixed with biotin-tagged antibody ~~mark~~marker at 25℃ for 2 hours. Then the ~~enzyme labeled avidin~~avidin-tagged enzyme (e.g., horseradish peroxidase, one that cleave or react against a detection substrate) was added into the ~~batten~~plate and incubated for 30 minutes. After adding the substrate ~~constant~~for the enzyme for 30 minutes, the ~~batten was added with the stop liquor~~stop solution was added to the plate. The mixed ~~liquor~~liquid was measured using the OD value at the

wavelength of ~~the~~ 450nm. The content of the TNF-α(ng/ml) was calculated based on the ~~data of~~ OD value ~~by the method of~~compared with standard curve.

The measurement of the IL-2:

The CTLL (cytotoxic T lymphoid cells) cells which ~~was~~were on the logarithmic growth phase and whose growth depends on ~~the~~ IL-2, were adjusted ~~to the~~in a suspension ~~at the~~to a concentration of 1×10^5 cells/ml with ~~the~~ 5% FCS-RPMI 1640. Then the 96~~-hole~~-well cell culturing ~~batten were added~~plate was filled with the CTLL cell suspension ~~on the quantity of 100μl/hole. The supernates were added on the quantity of 100μl/hole and each sample was added to 3 holes. The samples cultured~~at a volume of 100μl/well. Each sample was added in triplicate. To measure the concentration of IL-2, the cultured suspensions were compared with ~~the~~ different dilutions of standard rHIL-2 ~~and~~(recombinant human interleukin 2) in the control sample (culture fluid) ~~to measure the IL-2.~~. All the samples were ~~cultured~~incubated in ~~the~~ 5% CO2 for 24 hours at 37℃. Six (6) hours before the end of the ~~culture~~incubation, all the samples were centrifuged and separated from the supernate. Each ~~hole were taken out~~well had 110 μl of supernate removed and ~~added with~~then 10 μl of MTT was added to each well. The samples were cultured for 3 hours at 37℃, and then the OD was measured ~~the OD~~ at the ~~wavelength~~wavelengths of 570nm and 630nm. The final OD value of the sample was the difference of OD (570nm) and OD (630nm).

$$\text{IL-2 activity} = \frac{\text{Sample}\overline{OD} - \text{Control (Culture Fluid)}\overline{OD}}{\text{Standard Sample}\,\overline{OD} - \text{Control (Culture Fluid)}\overline{OD}} \times \text{activity of the standard sample (IU/ml)}$$

Table 5.1 The effect of Fengshiping on the TNF αnd IL-2 ($\overline{X} \pm S$)

group	dose (g/kg)	Mouse number	TNF (pg/ml)	IL-2 (IU/ml)
Control	-	10	87.80±14.63	26.30±4.22
Fengshiping	12	10	62.14±13.13**	16.00±2.89**
	24	10	58.60±9.63**	18.80±2.86**
	36	10	54.40±10.88**	18.20±2.86**
Tripterygium hypoglaucum (Levl.) Hutch.	8	10	58.25±10.32**	16.00±2.88**
cyclophosphane	0.02	10	42.20±9.57**	10.10±3.00**

*P<0.05 , **P<0.01

~~According to the~~The data in Table ~~5.1, it suggested~~5.1 suggest that ~~the~~ Fengshiping ~~have a~~has an obvious ~~inhibiting~~ inhibitory effect on the levels of TNFα. ~~On the~~At a dosage of 12g/kg, the medicine had ~~showed~~ ~~a~~shown an obvious ~~inhibiting~~inhibitory effect. ~~Along with the~~With an increase ~~of the~~in dosage, the ~~inhibiting~~inhibitory effect duly increased. But the dosage-effect curve ~~went gently. The~~was gradual. Fengshiping had an obvious ~~inhibiting~~inhibitory effect on the levels of IL-2, ~~ut~~but no dosage-effect relationship was observed.

5.2 The effect of Fengshiping on the IL-1, IL-6

Seventy (70) NIH mice, 18-22g weight, half male and half female, were divided into 7 groups at random. All the groups were ~~drenched with the correspondent~~given orally the corresponding medicines (~~fengshiping~~Fengshiping and ~~the~~ other medicines). The medicines were ~~drenched~~given once a day for 10 days. Twenty-four (24) hours after the last administration, all the mice were killed and ~~sampled the~~

~~macrophage~~macrophages and spleen cells from the abdominal carvity were sampled. The IL-1 and IL-6 in the samples were measured.

The measurement of IL-1:

The macrophages in the abdominal ~~carvity~~cavity were sampled in ~~the asepsis~~a sterile condition. Then the samples were washed ~~by the~~with Hank's ~~liquor for 2 times~~buffer twice and nonserum RPMI1640 ~~liquor for 1 time~~media once. Then the ~~clear~~washed samples were adjusted to ~~the~~ 4×10^6 cells / ml cell suspension with 5% FCS-RPMI ~~liquor~~media. One (1) ml of the suspension was added to the test tube and cultured at 37℃ for 1 hour. The ~~unadherent~~nonadherent cells were ~~abandoned~~discarded. Then ~~the cultured liquor was added with~~ 5% FCS-RPMI 1640 and LPS (10ng/ml) were added to cell culture. The cells were cultured in 5% CO_2 at 37℃ for 72 hours. ~~During the course~~Afterwards, the cultured cells were freezed and thawed ~~for~~ several times. The final product was saved at 4℃. ~~The~~Thymuses from C57 mice were ~~sampled the thymus in the asepsis~~obtained in sterile condition. Then the samples were ~~prepared~~adjusted to ~~the~~ 1×10^6 cells/ml cell suspension with 5% FCS-RPMI1640.

One hundred (100) μl supernate separated from the ~~frost thawing liquor~~freeze-thawed solution and 100μl cell suspension of the thymus were added ~~into the~~to 96-~~hole~~well flat bottom cell-culture ~~batten~~plates. Each sample was cultured in ~~3 holes~~triplicate and compared with the different dilutions of the standard rHIL-1 and the control sample (culture fluid). Each ~~hole was added with~~well had 2ng ConA added and then the ~~batten~~plate was cultured in ~~the~~ 5% CO_2 at 37℃ for 72 hours. Fourteen (14) hours before the end of the culture, each ~~hole~~well was added ~~with~~

3H-TdR (tritiated thymidine) at 0.1μCi. The cultured cells were collected with a multihead cell-harvesting apparatus and ~~measured~~ the cpm (count per minute) value was measured.

$$\text{IL-1activity} = \frac{\overline{\text{Sample cpm}} - \overline{\text{Control (Culture Fluid) cpm}}}{\overline{\text{Standard Sample cpm}} - \overline{\text{Control (Culture Fluid) cpm}}} \times \text{activity of the standard (ng/ml)}$$

The measurement of the IL-6:

The spleen cells were sampled in ~~the asepsis~~sterile condition. Then the samples were washed ~~by the~~ with Hank's ~~liquor for 2 times~~buffer twice and nonserum RPMI1640 ~~liquor for~~media 1 time. Then the clear samples were adjusted to ~~the~~ 2×10^6 cell/ml cell suspension with 5% FCS-RPMI ~~liquor~~media. One (1) ml of the suspension was added to ~~the~~a round-bottom centrifuge tube. After adding the ConA (10ng/ml), the samples were cultured in ~~the~~ 5% CO_2 at 37°C for 72 ~~hour~~hours.

The MH60 cells, ~~which grew depending on the~~their growth dependent on IL-6 and ~~were on~~at the logarithmic growth stage, were adjusted to ~~the~~ 1×10^5 cells/ml cell suspension with the 5% ~~FC~~FCS-RPMI1640.

The 96-~~hole~~well flat bottom cell culturing ~~batten~~plate was added ~~with~~ the MH60 cell suspension ~~on the quantity of~~at 100μl/~~hole~~well and the culturing ~~supernate~~suspension of spleen cells at 25μl/~~hole~~well. Then the ~~fluid~~volume in each ~~hole~~well was adjusted to ~~the~~ 200μl with ~~the~~ 5% FCS-RPMI 1640. Each sample was cultured ~~with 3 copies~~in triplicate and compared with ~~the~~solutions at different ~~solutions~~concentrations of standard rHIL-6 and the pure culturing fluid. The ~~batten~~plate was

~~cultured~~incubated in 5%CO_2 at 37℃ for 72 hours. Six (6) hours before the end of the ~~culture~~incubation, the samples were centrifuged. ~~Each hole~~ In each well, 110μl of the supernate was sucked out ~~the supernate 110μl~~ and 10μl of MTT was added ~~the MTT 10μl~~. The samples were kept at 37℃ for 3 hours. And then ~~they were measured~~ the OD at the wavelength 570nm and 630nm were measured. The final OD value = OD 570nm – OD 630nm.

$$\text{IL-6 activity} = \frac{\text{SampleOD - Culturing Fluid ControlOD}}{\text{Standard SampleOD - Culturing Fluid ControlOD}} \times \text{Sample Dilution} \times \text{Activity Of The Standard (IU/ml)}$$

Table 5.2 The effect of Fengshiping on the IL-1, IL-6 ($\bar{X} \pm S$)

Group	Dose (g/kg)	Mouse number	IL-1 (ng/ml)	IL-6 (IU/ml)
Control	-	10	78.7±7.1	94.6±6.8
Fengshiping	7.5	10	59.3±4.9**	64.9±4.8**
	15	10	53.3±5.7**	60.5±4.3**
	30	10	54.4±4.8**	56.0±4.6**
	60	10	47.0±16.6**	56.6±6.1**
Tripterygium hypoglaucum (Levl.) Hutch.	5	10	57.6±4.7**	65.7±4.9**
cyclophosphane	0.02	9	44.5±7.7	49.6±6.7**

Based on the data in the table 5.2, ~~the~~ Fengshiping had an ~~abvious inhibiting~~obvious inhibitory effect on the macrophage in producing ~~of~~ IL-1 and spleen cell in producing IL-6. ~~Along with the~~With increase ~~of the~~in dosage, the effect is duly enhanced ~~too~~.

5.3 The effect of Fenghsiping on the plasma NO (nitric oxide) in the AA

rat

Sixty (60) SD rats, 160 ~ 220g weight, half male and half female, were divided into 6 groups. The rats in the blank control group were injected ~~the NS~~with 0.5ml of NS (normal saline) under the skin of the right ~~postpede vola~~posterior hindlimb. Other rats were injected with ~~the~~0.5ml of FCA ~~0.5ml~~ at the same place as that of the control group. Eighteen (18) days later, the AA model was ~~built~~established. Then the rats were ~~drenched~~given orally the ~~correspondent~~corresponding medicines or ~~the~~ distilled water once a day for 5 days. Three (3) groups were ~~drenched~~given orally the solution of Fengshiping ~~on the~~ at high, middle and low dilution. The positive control group was ~~drenched with~~given orally Glucosidorum Tripterygll Totorum. The blank control group and the model group ~~were drenched with the~~was given orally distilled water of the same ~~volumn~~volume. One (1) hour after the last administration, 2 ml of each rat ~~was sampled the~~'s blood from the abdominal aorta ~~for 2 ml.~~was sampled. The plasma of the blood samples ~~were~~was separated and saved at - 70℃ for ~~the~~ measurement. The measurement of NO was done ~~on the direction of the NO reagent. 0.1ml plasma was added in 0.6ml reagent C and 0.4ml double distilled water. After the mixture shaken up, it was added in 0.1ml reagent D and cultured on the ice for 60 min. Then it was centrifuged at 12000 rpm for 2 min. The supernate was separated.0.6 ml supernate was mixed with 0.4ml double distilled water and 0.1ml reagent A, and then it was cultured in the ice water for 15 min. Then the mixture was added in reagent B 0.1ml and put at the room termperature for 1 hour. Then the new mixture was measured the OD at the wavelength 545nm~~as per the

directions of standard NO-detection kit. Based on the OD value of the sample, the content of NO was calculated on the standard curve. (See the result in table 5.3)

Table 5.3 The effect of Fengshiping on the plasma NO level in the AA rat ($\overline{X} \pm S$)

Group	Dose (g/kg)	Rat number	Content of NO (μmol/L)	y (y=Lgx)
Control	-	8	13.55±1.11*	1.131±0.032
AA model	-	9	17.56±4.15	1.235±0.097
Fengshiping	12	7	9.83±2.58**△△	0.985±0.087
Fengshiping	24	7	10.12±1.56**△△	1.001±0.067
Fengshiping	48	7	10.70±1.51**△△	1.026±0.062
Glucosidorum Tripterygll Totorum	0.006	7	15.25±3.48	1.173±0.099

Comparing to the model group*P<0.05 , **P<0.01 ; comparing to the Glucosidorum Tripterygll Totorum△△P<0.01

Based on the data in table 5.3, the NO level was higher in the model group than in the blank control group. ~~The~~ Fengshiping had an obvious effect on lowering the NO level in the AA rat. The Glucosidorum Tripterygll Totorum had ~~the~~ similar effect but its effect was weaker than that of ~~the~~ Fengshiping.

Experimental example 6 : The effect of Fengshiping on ~~the~~ T lymphocyte, CD_4, CD_8 and NK cells in the mouse.

6.1 The effect of Fengshiping on the ~~transform of lymphocyte~~transformation of lymphocytes in the normal mouse.

Eighty (80) NIH mice, half male and half female, were divided into 8 groups randomly and ~~drenched with the correspondent~~given orally the corresponding medicines once a day for 10 days. Twenty-four (24) hours

after the last administration, all the mice were killed to sample the spleen cells aseptically. Then the samples were washed ~~by the~~with Hank's ~~liquor for 2 times~~buffer twice and nonserum RPMI1640 ~~liquor for 1 time~~media once. Then the ~~clear~~washed samples were adjusted to ~~the~~ 2×10^6 cells/ml cell suspension with 5% FCS-RPMI ~~liquor~~media. The 96-~~hole~~well flat bottom cell culturing ~~batten~~plate was added ~~with~~ the cell suspension ~~on the quantity~~at a volume of 100μl/~~hole~~well. Each sample was cultured ~~with 3 copies. 2 holes~~in triplicate. Of the triplicate, two (2) wells were added ~~in~~ 2ng of ConA each as the stimulating reagent. The ~~other hole~~ third well was not ~~added in the~~ given ConA and was kept as the control ~~hole~~well. The ~~batten~~plate was ~~cultured~~incubated in 5% CO_2 at 37℃ for 72 hours. Fourteen (14) hours before the end of the ~~culture~~incubation, each ~~hole was added in~~well had 3H-TdR 0.1μCi added. The cells were harvested ~~by~~using the multihead cell harvesting instrument and were measured ~~the~~ for their cpm ~~value~~. The average value was adopted as the sample's cpm ~~value~~. The average ~~value~~cpm and the stimulating index of the different groups were compared directly. The stimulating index was calculated as ~~following~~follows:

$$\text{Stimulating Index} = \frac{\text{Stimulated } \overline{\text{cpm}}}{\text{Control } \overline{\text{cpm}}}$$

See the result in ~~tale~~table 6.1

Table 6.1 The effect of Fengshiping on the ~~lymphaeyto~~lymphocyte transformation induced by ConA in the mouse ($\overline{X} \pm S$)

Group	Dose (g/kg)	Mouse number	cpm	Stimulating index
Control	-	10	20433±3579	25.87±3.06
Fengshiping	7.5	10	13566±1779**	27.29±7.67
	15	10	12708±1692**	18.04±3.76

	30	10	12809±2575**	16.17±4.37
	60	10	12090±1706**	19.05±3.80
Tripterygium hypoglaucum (Levl.)	2.5	10	18038±3359	17.11±2.60
Hutch.	5	10	12081±1039**	17.58±4.37
Cyclophosphane	0.02	9	9922±1145**	13.66±2.28

Comparing to the control group*P<0.05 , **P<0.01

~~According to the~~The data in table ~~6.1, it indicated~~6.1 indicate that ~~the~~ Fengshiping had an obvious ~~inhibiting~~inhibitory effect on the lymphocyte transformation and there was a dosage-effect relationship.

6.2 The effect of Fengshiping on the CD_4, CD_8 and NK cells

The experiment was same ~~to~~as the experiment described in 5.1. Twenty-four (24) hours after the last administration, the spleen cell samples were made into ~~the~~a 2×10^8 cells/ml cell suspension with 5% FCS-RPIMl640. The quantity of CD_4, CD_8, NK cells and the rate CD_4/CD_8 were measured ~~on~~by the ~~usural~~usual method.

The measurement of CD_4 and CD_8:

~~The~~Fifty (50) μl of the spleen cell suspension ~~50μl~~ was added ~~on the~~to a glass to ~~made~~smear the cell ~~smear~~. The glass had been coated ~~by the~~with polylysine. The T cell of the mouse was set as the positive control sample. The cell smear was enveloped by the serum of the normal mouse after it was fixed ~~by the~~with acetone. Then the enveloped sample was ~~added~~incubated with ~~the antibody of~~antibodies to CD_4 and CD_8 ~~which were marked by the~~tagged with hominine biotin. It was incubated at 37℃ for 2 hours. Then ~~the sample was added with the avidin labeled by enzyme and put still~~avidin-tagged enzyme enzyme (one that can cleave or react against a detection substrate) was added to the sample and incubated for 10 min. After ~~added with~~ the substrate was added for 10 min, the mixed sample was washed and dyed with ~~the~~ hematoxylin for 2 min. Then the sample was ~~dyhidrated~~dehydrated with

the grade-alcohol and enveloped with gelatin-~~glycetrol.~~ glycerol. Two hundred (200) cells in the smear were chosen ~~as the research target~~to be evaluated under the high power microscope.

$$\text{Content Of Cell} = \frac{\text{Dyed cell number}}{200} \times 100\%$$

The measurement of the NK cell:

The preparation of the EC cell: The spleen cells were sampled in ~~the asepsis~~sterile condition. Then the samples were washed ~~by the~~ with Hank's ~~liquor for 2 times~~buffer twice and nonserum RPMI1640 ~~liquor for 1 time~~media once. Then the clear samples were adjusted to ~~the~~ 2×10^8 cells/ml cell suspension with 5% FCS-RPMI ~~liquor~~media. This cell suspension was used as the EC.

The preparation of the TC cell: The Yack-1 cells, which were sensitive to the mouse NK cell and ~~on~~growing at the logarithmic ~~growth~~ phase, were adjusted to ~~the~~ 4×10^4 cells/ml cell suspension. ~~It~~This cell suspension was used as the TC.

Measurement: EC and TC, One hundred (100) μl each were added ~~in~~to the 96-~~hole~~well flat bottom cell culturing ~~batten~~plate. Each sample was cultured in triplicate with ~~3 copies and set~~ 2 control samples: EC and TC. (EC control: EC100μl + 5% FCS RPMI 1640 100μl ; TC control : TC100μl + 5% FCS RPMI 1640 100μl). The samples were ~~cultured~~incubated in 5% CO_2 at 37℃ for 24 hours. Six (6) hours before the end of the ~~culturing~~incubation, the samples were centrifuged and ~~sucked out~~ 110μl supernate were aspirated out of each ~~hole~~well. And then ~~the holes~~10μl of MTT were added ~~in the MTT 10μl.~~to each well.

After ~~put~~incubating at 37℃ for 3 hours , the OD values of the mixed samples were measured ~~the OD value~~ at the ~~wavelength~~wavelengths of 570nm and 630 nm. The OD of each ~~hole~~well =OD570nm - OD630nm.

$$\text{Activity Of NK} = \left(1 - \frac{\text{Sample } \overline{OD} - \text{EC Control } \overline{OD}}{\text{TC Control } \overline{OD}}\right) \times 100\,\%$$

Table 6.2 The effect of Fengshiping on the CD4, CD8, NK cell ($\overline{X} \pm S$)

Group	Dose (g/kg)	Mouse number	CD4 (%)	CD8 (%)	CD4/CD8	NK
Control	-	10	20.80±2.94	14.80±2.49	1.42±0.18	40.13±4.89
Fengshiping	12	10	19.14±2.91	13.43±2.51	1.43±0.08	31.94±4.52**△△
	24	10	17.30±2.51**	12.00±2.40	1.46±0.16	35.36±3.40*△△
	36	10	16.30±2.50**	11.23±2.94**	1.49±0.20	31.06±3.53**△△
Tripterygium hypoglaucum (Levl.) Hutch.	8	10	16.25±2.25**	11.50±2.45	1.44±0.18	32.20±2.00**
Cyclophosphane	0.02	10	11.50±2.50**	4.10±1.20**	2.91±0.53**	23.10±3.66**

Comparing to the control group*P<0.05，**P<0.01；comparing to the cyclophosphane△△P<0.01

According to the table 6.2, ~~it ie~~Fengshiping had some inhibitory effect on CD_4 cells and CD_8 cells. There was a relation between the ~~dzosage~~dosage and the effect, but the dosage-effect curve was ~~smooth~~gradual. The effective dosage of Fengshiping on the ~~inhibiting~~inhibition of CD_4 was 24g/kg. The minimum effective dosage on inhibiting the CD_8 was 36g/kg. ~~As the rate of CD_4/CD_8, the~~ Fengshiping had no obvious effect~~.~~ on the ratio of CD_4/CD_8. Cyclophosphane had an obvious effect ~~on the~~in inhibiting ~~of the~~ both kind of cells~~, and~~ with the ~~inhibiting~~inhibitory effect on the CD_8 was very ~~powerful~~potent, which could increase the ~~rate~~ratio of CD_4/CD_8 ~~magnificently~~significantly.

As for NK cell, ~~the~~ Fengshiping had a remarkable ~~inhibiting~~inhibitory effect, but the dosage-effect relationship was not certain. ~~As the same while, the~~Similarly, cyclophosphane had shown an obvious ~~inhibiting~~inhibitory effect on the NK cell. ~~On the~~At a dosage of 20mg/kg, the ~~inhibition~~inhibitory effect of cyclophosphane was significantly different from that of ~~the~~ Fengshiping ~~on the~~at its 3 dosages: 12, 24 and 36g / kg.

6.3 The effect on the transformation and function of the T ~~lymphacyto~~lymphacyte in the AA mouse.

NIH mice, 20±2g weight, were injected with 0.05 ml of FCA under the skin of the right ~~postpede vola~~posterior limb to build the AA model. The mice in the control group were injected with 0.05ml NS at the same place. Three (3) weeks later, after the AA model was built, all the mice were ~~drenched~~given orally the ~~correspondent~~corresponding medicines once a day for 5 days. Five (5) days later, all the mice were sampled, and

the blood was used to make ~~the~~ blood ~~smear~~smears. The smears were dyed by ~~the~~ esterase. Then the smears were observed under ~~the~~an oil immersion lens to calculate the percent of the positive-dyed cells (it represented the content of the T cells in the blood). The spleen cells of the mice were sampled ~~the spleen cells in the condition of~~while the mice were under anaesthesia and then the cell samples were prepared ~~to the single~~in a cell suspension. The cell suspension was washed by PBS and then its supernate ~~were abandoned~~was discarded. The rest ~~part was added with~~had 4 ml of blood ~~cytolysate 4ml~~cell lysate added. The mixed sample was ~~shaked~~shaken for 2 ~ 3 min to ~~solute~~solubilize the RBC. After the RBCs were ~~destroyed~~solubilized, the sample was centrifuged to separate and ~~abandon~~discard the supernate. The sample without supernate was washed ~~by~~with the luminescence ~~lotion for 2 times~~solution twice. Then it was centrifuged to separate and ~~abandon~~discard the supernate. In the next step, the sample was adjusted to the 1×10^6 cells/ml cell suspension. Each tube was added ~~with~~ 50μl of diluted antibody ~~of~~to CD_4 and CD_{8}. Then the ~~tubs~~tubes were ~~cultured~~incubated at 4℃ for 1 hour. After the culture, the samples were washed with the luminescence ~~lotion for 2 times~~solution twice and ~~added in~~2ml of the fixing fluid ~~2 ml~~ was added. After fixing, the samples were filtrated through the 400-mesh screen to the FCA tube. The filtrated samples were analyzed by ~~the~~ flow cytometer (FCM). The result ~~was~~is shown in ~~the~~ table 6.3.

Table 6.3 The effect of Fengshiping on the T cell in the AA mouse

$(\overline{X} \pm S)$

Group	Dose	ANAE+	CD4+	CD8+	CD4+/CD8+

	(g/kg)	(%)	(%)	(%)	
Control	-	50.60±4.25	26.13±1.16	15.56±0.68	1.68±0.03
AAmodel	-	49.00±4.22▲	32.56±2.87**	13.59±1.03**	2.49±0.16**
	7.5	49.13±4.03▲	27.30±1.76##▲	15.98±1.11##▲	1.71±0.04##▲
Fengshiping	15	49.31±3.29▲	27.96±1.67##▲	16.23±1.27##▲	1.73±0.05##▲
	30	48.56±3.23▲	26.75±1.94##▲	15.58±1.29##▲	1.72±0.04##▲
Glucosidorum Tripterygll Totorum	0.012	48.88±2.89▲	27.88±1.99##▲	16.33±1.31##▲	1.70±0.03##▲

n=8 , comparing with the control group*P<0.05 , **P<0.01 ; comparing with the model group# P<0.05 , ## P<0.01 ; comparing with the control group▲P>0.05

According to the data in table 6.3, there was no significant difference in the different groups on the ANAE (alpha- naphthyl acetate esterase) positive cell. But in the AA mouse, the increase of the CD_4 was significant, while the decrease of CD_8 was significant too. So the ~~rate~~ratio of CD_4/CD_8 had a remarkable increase. The result ~~indicated~~indicates that ~~the~~ Fengshiping could adjust the CD_4, CD_8 and CD_4/CD_8 to the normal range.

Experimental example 7: The effect of Fengshiping on the phagocytic function of the macrophage in the mouse abdominal carvity.

Fifty (50) NIH mice, 18~ 22g weight, half male and half female, were divided into 5 groups and ~~drenched with the correspondent~~ given orally the corresponding medicine solutions ~~on~~at the same ~~volumn~~volume. The administration was once a day for 7 days. One (1) hour after the last administration, all the mice were injected with 0.2ml 10 % chick RBC into the abdominal ~~carvity~~cavity. Four (4) hours later, all the mice were killed and ~~sampled~~ the fluid in the ~~abdominal carvity~~abdominal cavity was sampled. The ~~liquor~~liquid samples were

~~dropped~~deposited on the glass and ~~counted~~ the number of ~~the macrophage which~~macrophages was counted that had phagocytized the CRBC and the number of the CRBC in ~~one~~each macrophage was also counted. (See the result in table 7)

Table 7 The effect of Fengshiping on the CRBC phagocytosis function of the macrophage in ICR mouse abdominal cavity ($\overline{X} \pm S$)

group	dose (g/kg)	Mouse number	Percent of phagocytosis (%)	phagocytosis index
Control	-	10	25.75±9.40	1.28±0.20
Fengshiping	27	10	33.20±12.77	1.46±0.36
Fengshiping	40.5	10	35.20±10.16	1.21±0.20
Fengshiping	60.9	10	37.78±20.14	1.53±0.32
dexamethasone	0.005	10	8.33±10.13*	1.10±0.18

*P<0.05

According to the table 7, ~~the~~ Fengshiping had no obvious effect on the ~~phagocytosis~~phagocytotic function of the macrophage in the mouse abdominal cavity.

Experimental example 8: The effect of Fengshiping on the hyperfunction of the capillary permeability in the mouse abdominal cavity.

Ninety (90) NIH mice, 18~22g weight, half male and half female, were divided into 9 groups and ~~drenched with~~given orally the

~~correspondent~~corresponding medicine solutions of the same ~~volumn~~volume. The medicines were ~~drenched~~given once a day for 3 days or just 1 time. One (1) hour after the last administration, each mouse ~~were~~was injected with 0.7% HAC (acetic acid) – NS solution into the abdominal cavity. At the same time, each mouse was injected with the 0.5% Evans blue – NS solution into the vessel ~~on the~~at a dose of 0.1ml/10 g. Thirty (30) min later; all the mice were killed by cervical ~~disjoint~~dislocation. The abdominal cavity was opened and washed ~~by the~~with 5ml NS. The NS used was collected and adjusted to a volume of 8ml by ~~the~~ pure NS to be used as the sample. The samples were centrifuged at 3000 rpm to get the supernate. The supernate OD was measured ~~the OD~~ at the wavelength ~~at~~of 590nm. (See the result in table 8)

Table 8 The effect of Fengshiping on the hyperfunction of the capillary permeability induced by the acetic acid in the mouse abdominal cavity ($\overline{X} \pm S$)

Group	Dose (g/kg)	Administration	Mouse number	Leakage of the tincture (OD)	P value
Control	-	-	10	0.29±0.13	
Fengshiping	27	qd×1	10	0.26±0.14	>0.05
Fengshiping	40	qd×1	10	0.25±0.10	>0.05
Fengshiping	60	qd×1	10	0.25±0.09	>0.05
Control	-	-	10	0.28±0.15	
Fengshiping	27	qd×3	10	0.25±0.12	>0.05
Fengshiping	40	qd×3	10	0.18±0.10	<0.05
Fengshiping	60	qd×3	10	0.15±0.13	<0.05
dexamethasone	0.15	qd×3	10	0.11±0.07	<0.01

~~According to the~~The data in table ~~8, it indicated~~8 indicate that Fengshiping could obviously inhibit the hyperfunction of the capillary permeability induced by the acetic acid in the mouse abdominal cavity if

~~it~~the mouse was ~~drenched~~given the medicine for 3 days continuously. If the medicine was ~~drenched~~given for just ~~1 time~~once, the inhibiting effect was not obvious.

Experimental example 9: The effect of Fengshiping on the pleuritis exudation and aggregation of the inflammatory cell ~~aggregation~~ induced by the carrageenan.

The mice were divided into 5 groups at random and injected with 0.5% Evans blue NS solution into the caudal vein ~~on the~~at a dosage of 0.1ml/10g. Then the mice were injected with the 0.03ml 1% carrageenan in the right chest cavity with ~~the special syring niddle. 4~~a syringe needle. Four (4) hours and 32 hours after the injection, the ~~correspondent~~corresponding mice were killed and ~~opend the obdominal~~had their abdominal cavity opened to expose the diaphragm. Two (2) ml of the ~~lotion~~solution were injected to the chest cavity ~~by 2 times~~twice with a 1 ml injector. The ~~lotion~~solution was collected and saved in a test tube. Twenty (20) μl of the ~~lotion~~solution collected was added into the 400μl WBC dilution. The WBC in the mixed dilution was counted under the microscope. The rest of the ~~lotion~~solution was centrifuged at 3000rpm for 10 min. The supernate ~~of the lotion~~ was measured ~~the~~for its OD at ~~the~~a wavelength of 600nm. The OD value of the sample should be corrected with the correspondent OD value of the pure ~~lotion~~solution. (See the result in table 9)

Table 9 The effect of Fengshiping on the inflammatory cell aggregation induced by the carrageenan ($\overline{X} \pm S$)

Group	Dose (g/kg)	WBC number(2×105)		Tincture exudation (OD)	
		4h	32h	4h	32h

Control	-	46.0±6.9	16.0±9.6	0.156±0.066	0.109±0.019
Fengshiping	27	26.8±4.5*	14.2±8.0	0.121±0.062	0.116±0.031
Fengshiping	40.5	10.9±4.0**	17.3±4.6	0.100±0.048	0.153±0.032
Fengshiping	60	8.0±5.5**	6.6±4.7*	0.129±0.066	0.092±0.051
dexamethasone	0.05	12.7±10.2**	4.4±4.0*	0.085±0.045	0.063±0.017

*P<0.05 , **P<0.01

~~According to the~~The data in table ~~9, it indicated~~9 indicate that ~~the~~ Fengshiping had an obvious ~~inhibiting~~inhibitory effect on the inflammatory cell aggregation. The effect was powerful at the early stage. The regression equation on the data of the fourth hour was as ~~following~~follows: y=44.13 - 2.01x , r= - 0.9625. The effect ~~on~~at the late stage was weak. At the high dosage of 20g/kg, the medicine could affect the aggregation of the WBC. But it had no obvious effect on the pleuritis exudation.

Experimental example 10: Effect on aggregation of ~~leucocyte~~leukocyte in rats~~'~~ with a sac of CMC ~~sac~~(carboxymethylcellulose).

Sixty four (64) SD rats, 150-180g weight, half male and half female, were randomly divided into 8 groups, ~~which~~and they were ~~drenched with~~given orally the same volume ~~and~~but different dosage of drug liquid once a day~~, lasting~~ for 3 days. A day before experiment, rats were injected with 20ml 1% CMC solution into the sac at the rat's back ~~caused~~created by 20ml air injection before the experiment. ~~3.5 hour~~ Three and a half hours and 7.5 ~~hour~~hours later, 0.1ml of liquid in the sac was extracted each time, and was colored ~~in~~with 0.01% brilliant cresyl blue solution~~. leucocyte was~~ and leukocytes were counted in the sac ~~liquor~~liquid under a microscope. The results ~~showed~~are shown in the table 10.

Table 10 effect on leucocyte counts of carboxymethyl cellulose sac of rats with Fengshiping ($\overline{X} \pm S$)

groups	dosage (g/kg)	rats number	WBC count(×107/L) 3.5 hrs	WBC count(×107/L) 7.5 hrs
control	-	8	9.7±4.2	57.7±17.3
Fengshiping	27×1	8	8.5±3.5	39.4±16.5
Fengshiping	40×1	8	8.7±7.3	35.3±23.2
Fengshiping	60×1	8	6.6±3.3	18.1±8.6**
Control	-	8	10.97±6.7	35.6±11.2
Fengshiping	27×3	8	15.4±9.7	38.6±15.5
Fengshiping	40×3	8	4.8±3.4**	18.4±12.2**
Fengshiping	60×3	8	3.0±2.8**	11.0±9.2*
cortisone	0.1×3	8	14.2±8.0	41.7±16.0
Control	-	8	10.9±3.0	41.3±6.9
Fengshiping	18×7	8	6.2±3.0*	11.4±6.4*
Fengshiping	27×7	8	3.7±1.7**	6.4±3.1**
Fengshiping	40×7	8	2.5±1.9**	5.9±3.9**
cortisone	2mg×1	8	1.5±0.7**	3.0±1.0**

Compared with control group**P<0.01

According to ~~the~~ table 10, ~~the~~ Fengshiping could inhibit significantly aggregation of leucocyte in the rats' ~~CMC~~ sac containing CMC, and the inhibition showed apparent dosage-effect ~~relation~~correlation, which was stronger ~~as~~with greater frequency of administration ~~time lasted~~. With administration ~~of~~ continuing seven days, ~~wandering~~the migration of leucocyte could be inhibited significantly at dosage of 18g/kg~~, at the same time~~. Similarly, there was also very strong inhibition with cortisone injection into the sac.

Experimental example 11: The effect on croton oil-induced swelling in the ears of mice.

Sixty (60) NIH mice with weight of 18~22g, male and female accounting for half and half, were divided into 6 groups, which were ~~drenched~~given orally with the same volume and different dosage of drug liquid or tragacanth liquid, once a day, lasting 3 days. One (1) hour after

the final administration, 2% croton oil mixure of 0.02ml was embrocated uniformly on ~~the~~ both sides of the left ears of the mice, and after 4 hours, the mice were ~~snapped off its~~put to death by snapping their cervical vertebra ~~and put to death~~. The left and right ears were cut down, then ~~inflammatory~~the inflammed and control ears were ~~weighted~~weighed by certain means. ~~Difference of~~Differences in weight between left and right ears ~~was~~reflect the ~~swelling~~ extent of the swelling of the inflammed ears, with results ~~showing~~shown in table 11.

Table 11 ~~effect~~Effect on croton oil-induced swelling of the ears of mice with Fengshiping ($\overline{X} \pm S$)

Groups	dosage (g/kg)	rats number	Degree of ears' swelling (mg)	inhibition rate (%)	P value
Control group	-	10	44.38±9.40		
Fengshiping	27	10	39.05±12.33	12.00	>0.05
Fengshiping	40	10	36.65±5.83	17.64	<0.05
Fengshiping	60	10	34.91±9.71	21.34	<0.05
dexamethasone	0.003	10	14.13±5.75	68.16	<0.01

~~It was~~As seen from table 11, ~~that~~ Fengshiping had remarkable inhibition to croton oil-induced swelling of the ears of mice, and had ~~quantity~~dosage-effect ~~relation~~correlation, but ~~which~~the curve was ~~gentle and smooth~~gradual. There was significant inhibition effect at 13.5g/kg of dosage.

Experimental example 12: Effect on acetic acid-induced twisting reaction of mice.

Sixty (60) Kuming mice with weight of 18~22g, male and female accounting for half and half, were randomly divided into 6 groups, which were ~~drenched~~given orally different dosages of drug liquid or ~~Xihuangqi~~tragacanth solution. One (1 ~~hours~~) hour after administration, 0.2ml of 0.7% HAC saline ~~of 0.2ml~~solution was injected, sc

(subcutaneous), and the mice were placed in ~~aquarium and obervedan~~ enclosure and observed for the length of the latent period before the twisting reaction of each mouse and the twisting times in 20 minutes~~,~~ with the results ~~showing~~shown in table 12:

Table 12 The effect of Fengshiping on acetic acid-induced twisting reaction of mice ($\overline{X} \pm S$)

groups	dosage (g/kg)	Rats numbers	Twisting times	Latent time (minute)
Control	-	10	34.6±14.1	3.13±0.80
Fengshiping	27	10	28.2±5.76	3.82±0.85
Fengshiping	40	10	31.0±18.4	3.86±2.00
Fengshiping	60	10	20.7±12.3*	3.95±1.42
Tripterygium hypoglaucum (Levl.) Hutch.	20	10	25.1±11.9	3.60±0.93
morphine hydrochloride	10mg/kg	10	0.0±0.0	0.00±0.00

~~It was seen~~The data from table 12 indicate that large ~~dose~~doses of Fengshiping could delay the latent time before the HAC-induced twisting reaction and significantly reduce the twisting times in 20 minutes, which indicated Fengshiping had the effect of ~~abirritation~~aberration in some degree.

Experimental example 13: Effect on hemorheology of AA rats.

Each ~~of~~ SD (Sprague Dawley) rats, 180±20g weight, were injected intracutaneously with 0.05ml Freund's complete adjuvant on the right back foot metatarsal, and they were developed into adjuvant arthritis model~~s~~. Each of the rats of negtive control group were injected intracutaneously with 0.05ml ~~salin~~saline on the right back foot metatarsal. Three weeks after models were built, the rats were divided into model group, large, middle, small dosage ~~group~~groups, negtive control group and positive control group which was administered with Glucosidorum Tripterygll Totorum. The rats were ~~drenched~~given the

medicines orally once a day, lasting 5 days~~, 1 hours~~. One (1) hour after administration for the last time, ~~and~~ 3ml of blood was taken from abdominal aorta of rats and placed into test tube with 1% heparin as ~~decoagulant, in which~~an anticoagulant, and the whole blood viscosity was measured at shear ~~rate~~rates of 230, 115, 46, 23, 11.5, 5.75S^{-1} with an NXE-1 cone and plate viscometer. The plasma viscosity was measured with a WTP-BII adjustable constant pressure capillary viscosimeter. The haematocrit, erythrocyte aggregation index was measured with the centrifugation method of packed cell volume. The rigidity index was calculated from the above-mentioned data. All the results ~~showed~~are shown in table 13.

Table 13 Effect on hemorheology of adjuvant arthritis model rats ($\bar{X} \pm S$)

Groups	Control group	Model group	Fengshiping (30g/kg)	Fengshiping (15g/kg)	Fengshiping (7.5g/kg)	Glucosidorum Tripterygll Totorum (6mg/kg)
whole blood viscosity (mPa.s)						
230S-1	4.43±0.09	4.92±0.15**	4.56±0.09##	4.49±0.11##	4.54±0.16##	4.66±0.28#
115S-1	5.17±0.25	5.81±0.19**	5.33±0.09##	5.32±0.10##	5.16±0.14##	5.60±0.48#
46S-1	6.84±0.11	7.20±0.18**	6.56±0.13##	6.59±0.09##	6.67±0.14##	6.70±0.48#
23S-1	8.10±0.15	8.23±0.38	7.95±0.22	7.93±0.12	7.97±0.14	8.02±0.14
11.5S-1	9.35±0.08	9.78±0.10**	9.40±0.08##	9.45±0.10##	9.30±0.133	9.31±0.12##
6.5S-1	11.03±0.14	12.66±0.31**	11.21±0.21##	11.29±0.19##	11.60±0.40##	11.42±0.52##
Plasma viscosity (mPa.s)	1.158±0.032	1.248±0.040**	1.161±0.011##	1.154±0.023##	1.156±0.018##	1.158±0.029##
corpuscular volume (%)	46.13±2.31	41.33±1.12**	45.10±2.39##	44.33±1.52##	45.71±1.04##	46.03±3.59##
erythrocyte aggregation index	2.49±0.032	2.58±0.083*	2.46±0.066#	2.49±0.094#	2.44±0.048##	2.45±0.091#
Rigidity index	6.155±0.536	7.127±0.557**	6.506±0.558	6.525±0.146	6.394±0.200#	6.621±0.883

Compared with negative control group*P<0.05 , **P<0.01 ; compared with model control group# P<0.05 , ## P<0.01

According to the table 13, the hemorheology of AA rats ~~were~~was changed significantly compared with control rats. The whole blood and plasma viscosity increased, haematocrit decreased, aggregation index and ~~rigity~~rigidity index of erythrocyte increased. ~~The~~ Fengshiping could ~~make~~significantly improve the above-mentioned indexes of hemorheology ~~improved significantly~~.

Pharmacological effects of Fengshiping have been ~~proved~~proven by the above-mentioned experiments. Many important pharmacological effects of Fengshiping had favorable dosage-effect ~~relation~~correlation, which implied the best therapeutic effectiveness might be obtained by adjusting the drug dosage at the clinical ~~work~~level.

The clinical studies on Fengshiping were carried on in China, Japan and Austrilia. Theses studies were ~~operated~~done according to international criterion related disease classification ~~about~~regarding diagnosis, therapy and curative effect. ~~By using the Fengshiping capsules Sololy, its~~The effective rate for RA was around 94%, and its ~~remarkable~~notable effective rate was around 60%. It could improve the symptoms such as morning stiffness, swelling and pain and ~~so on and the~~other related items. The results showed in ~~table~~tables 14 ~ 21.

Table 14 Compared effect of treatment group with control group

Groups	Cases	remission (clinical recovery)	Notable effect	Effective	No effect	Notable effect rate (%)	Effective rate (%)
Treatment group	32	5	14	11	2	59.38	93.74
Control group	30	3	10	12	5	43.33	83.33

Table 15 Influence of IgG, IgA and IgM ($\overline{X} \pm S$)

Groups	cases	IgG		IgA		IgM	
		pre -	post -	pre -	post -	pre -	post -
Normal	32	12.45±1.48		2.37±1.00		1.58±0.59	
Treatment group	32	16.92±3.49	14.17±1.39**	3.65±1.03	2.39±1.18**	1.89±0.88	1.48±1.01
Control	30	17.03±4.12	15.14±2.21**	3.45±1.86	2.32±1.75**	2.03±0.95	1.76±1.28

Comparing with pre-treatment **P<0.01

Table 16 Influence of C3 and C4($\overline{X} \pm S$)

groups	cases	C3		C4	
		pre -	post -	pre -	post -
normal group	32	0.62±0.13		0.14±0.15	
Treatment group	32	1.88±0.72	1.25±0.66**	0.48±0.12	0.26±0.06*
Control group	30	2.13±0.64	1.56±0.62**	0.40±0.16	0.25±0.07**

Comparing with before therapy *P<0.05, **P<0.01

Table 17 Influence of ESR and CRP ($\overline{X} \pm S$)

Groups	cases	ESR		CRP	
		pre-	post-	pre-	post-
Normal	32	8.37±5.26		4.12±1.88	
Treatment	32	66.58±9.01	30.31±6.53**	13.35±6.67	8.86±3.34*
control	30	73.33±9.09	35.83±11.61**	14.21±6.29	9.04±3.15**

Comparing with pre-treatment *P<0.05, **P<0.01

Table 18 Compared with power of gripping pre- and post-treatment ($\overline{X} \pm S$)

groups	Treatment Group		Control Group	
	pre -	post -	pre -	post -
Gripping power of left hands (mmHg)	39.13±20.24(15)	80.47±34.61**(15)	24.00±17.63(21)	55.15±23.27**(21)
Right hands	35.85±22.46(15)	85.32±36.32**(15)	22.80±12.32(21)	58.17±20.59**(21)

Comparing with pre-treatment *P<0.05, **P<0.01

Table 19 Influence of arthrosis swelling and pain and morning stiffness time ($\overline{X} \pm S$)

Items	Treatment Group		Control Group	
	pre -	post -	pre -	post -
arthrosis swelling and pain	5.79±0.52	3.14±0.83*	5.56±2.15	3.92±0.26*
morning stiffness time (minute)	50.33±6.47	20.24±3.27**	48.75±8.34	27.50±3.78**

Comparing with pre-treatment *P<0.05, **P<0.01

Table 20 Influence of RF changing to negative

Groups	Cases	RF negative		
		Pre - treatment	Post - treatment	Rate of negative turnaround (%)
Treatment group	32	24	11	54.2
Control group	30	18	10	44.4

Not only ~~had~~did it show significant effects~~,~~ for the above items in tables 14-20, but ~~also~~ Fengshiping can ~~make~~also decrease the levels of items such as SIL-2R, STNF, SIL-6R in plasma ~~decrease, results showing~~as shown in the Table 21.

Table 21 inflence of main indes such as SIL - 2R, STNF and SIL - 6R ($\overline{X} \pm S$)

groups	Cases	SIL - 2R(u/ml)		STNF R1(ng/ml)		SIL - 6R(ng/ml)	
		pre -	post -	pre -	post -	pre -	post -
Normal	32	299±68 (n=32)		1.56±0.48 (n=24)		72.05±18.26 (n=22)	
Fengshiping	15	683±189	381±157**	2.87±0.66	1.75±0.54**	136.18±28.57	90.15±20.12**
Control	10	765±203	412±167**	2.63±0.72	2.38±0.39 (n=8)	148.21±30.31	99.02±26.70**

Comparing with pre-treatment **P<0.01

It was ~~proved that~~proven in the above-mentioned results ~~on~~that the

invention ~~could~~can be realized ~~on~~by the ways as ~~following.~~follows~~:~~:

~~Practice example 1:~~

Example of use 1:

Epimedium brevicornum Maxim. 2222g

Tripterygium hypoglaucum (Levl.) Hutch. 2222g

Lycium barbarum L. 1111g

Cuscuta chinensis Lam. 1111g

~~Four herbs hereinbefore,~~ *Tripterygium hypoglaucum* (Levl.) Hutch. was cut into pieces, extracted for three times ~~after~~with 13, 10, 10-~~fold added in~~volume water , each time lasting 1 hour; *Epimedium brevicornum* Maxim was cut into segments, extracted three times ~~after~~with 15, 10, 10-~~fold~~volume water ~~was added in~~, each ~~extraction~~time lasting 1 hour; *Lycium barbarum* L. was crushed ~~to raw material~~into coarse powder, and immersed in 20-~~fold~~volume water of 80℃ for 1 hour; *Cuscuta chinensis* Lam. was crushed ~~to raw~~into coarse powder, immersed in 31-~~fold~~volume water of 80℃ for 1 hour; the decoction fluid or immersion fluid of four herbs were filtrated ~~repectively~~respectively, poured ~~across macropore~~into column with polymeric adsorbent ~~column,~~resins having macropores, and eluted with 70% ethanol. When the color of ~~effluent~~eluent became deep significantly, eluent was ~~commenced to collect; when~~collected until the color of ~~effluent~~eluent became very weak, at which time the elution collection ~~was~~ ended. ~~Eluent~~The alcohol in the eluent of each herb was ~~recycled to get ethanol~~reduced. Then the fluid without alcohol was concentrated~~,~~ and dried to get the ~~finally extractive drug~~final extract powder; officinal starch was blended with the four kinds of ~~drug~~extract powder to 200g, mixed ~~up~~ uniformly and encapsuled into 1000 capsules. Each capsule

which was prepared with the invented method thereof, was composed of 0.2g ~~drugs extractive~~drug extract and contained at least 2.0mg of ~~icariine~~icariin $C_{33}H_{40}O_{15}$. The regular dosage is: oral administration, three times every day, three capsules ~~every~~each time.

~~Practice example 2:~~

Example of use 2:

Tripterygium hypoglaucum (Levl.) Hutch.2000g

Epimedium brevicornum Maxim.2000g

~~Two herbs hereinbefore,~~ *Tripterygium hypoglaucum* (Levl.) Hutch. ~~were~~was cut into pieces, extracted three times ~~after 13, 10, 10-fold added in~~with 13, 10, 10-volume water, each time lasting 1 hour; ~~Epimedium brevicornum Maxim.~~*Epimedium brevicornum* Maxim. was cut into segments, extracted three times ~~after 15, 10, 10-fold water was added in, each extraction~~with 15, 10, 10-volume water, each time lasting 1 hour; decoction fluid of herbs were filtrated ~~repectively~~respectively, poured ~~across macropore~~into column with polymeric adsorbent ~~column~~resins with macropores, eluted with 70% ethanol, when the color of ~~effluent~~eluent became deep significantly, eluent was ~~commenced to collect; when the color of effluent became very weak, elution was over. Eluent of each herbs was recycled to get ethanol, concentrated, dried, finally extractive drug powder was obtained~~collected until eluent became very weak, at which time the elution ended. The alcohol in the eluent of each herb was removed. Then the fluid without alcohol was concentrated, dried to get the final extract powder; officinal starch was blended with the ~~extractive~~extracted drug powder, and mixed ~~up~~ uniformly, loaded to 1000 capsules. Each capsule which was prepared with the inventive method thereof, is composed of 0.2g drugs extractive,

contains at least 2.0mg of ~~icariine~~icariin $C_{33}H_{40}O_{15}$. regular dosage is: oral administration, three times every day, three capsules ~~every time.~~for each time.

~~Practice example 3:~~

Example of use 3:

Tripterygium hypoglaucum (Levl.) Hutch.2000g

Epimedium brevicornum Maxim.2000g

Lycium barbarum L. 1000g

Tripterygium hypoglaucum (Levl.) Hutch. ~~were~~was cut into pieces, extracted three times ~~after~~with 13, 10, 10-~~fold added in~~times water, each time lasting 1 hour; *Epimedium brevicornum* Maxim.was cut into segments, extracted three times ~~after 15, 10, 10-fold water was added in, each extraction~~with 15, 10, 10-times water, each time lasting 1 hour; *Lycium barbarum* L. was crushed to ~~raw material~~coarse powder, and immersed in 20-~~fold~~times water of 80℃ for 1 hour; decoction fluid or immersion fluid of four herbs were filtrated ~~repectively~~respectively, poured across a macropore polymeric adsorbent column, eluted with 70% ~~ethanol~~alcohol, when the color of effluent became deep significantly, eluent was ~~commenced~~started to ~~collect~~be collected; when the color of effluent became very weak, elution was over. ~~Eluent of each herbs was recycled to get ethanol,~~The alcohol in the eluent of each herb was recovered. Then the fluid without alcohol was concentrated, dried~~,~~ to get the finally ~~extractive drug powder was obtained~~extract powder; officinal starch was blended with the extractive drug powder, and mixed up uniformly, loaded to 1000 capsules. Each capsule which was prepared with the inventive method thereof, is composed of 0.2g drugs extractive, contains at least 2.0mg of icariine $C_{33}H_{40}O_{15}$. Regular dosage is: oral administration, three times every day, three capsules ~~every~~each time.

~~Practice example 4~~

Example of use 4:

Tripterygium hypoglaucum (Levl.) Hutch.2000g

Epimedium brevicornum Maxim.2000g

Cuscuta chinensis Lam. 1000g

Tripterygium hypoglaucum (Levl.) Hutch. ~~were~~was cut into pieces, extracted three times ~~after~~with 13, 10, 10-~~fold added in~~volume water , each time lasting 1 hour; *Epimedium brevicornum* Maxim.was cut into segments, extracted three times ~~after 15, 10, 10-fold water was added in, each extraction~~with 15, 10, 10-volume water, each time lasting 1 hour; *Cuscuta chinensis* Lam. was crushed to ~~raw~~coarse powder, immersed in 31-~~fold~~volume water of 80℃ for 1 hour; decoction fluid or immersion fluid of the herbs were filtrated ~~repectively~~respectively, poured across a macropore polymeric adsorbent column, eluted with 70% ethanol, when the color of effluent became deep significantly, collection of eluent ~~was commenced to collect~~began; when the color of effluent became very weak, elution was over. ~~Eluent of each herbs was recycled to get ethanol, concentrated, dried, finally extractive drug powder was obtained~~The alcohol in the eluent of each herb was reduced. Then the fluid without alcohol was concentrated, and dried to obtain the final extract powder; officinal starch was blended with ~~extractive~~extract drug powder, and mixed ~~up~~uniformly, loaded to 1000 capsules. Each capsule which was prepared with the inventive method thereof, is composed of 0.2g ~~drugs extractive~~drug extract, contains at least 2.0mg of ~~icariine~~icariin $C_{33}H_{40}O_{15}$. Regular dosage is: oral administration, three times every day, three capsules ~~every~~each time.

~~Practice example 5~~

Example of use 5:

Tripterygium hypoglaucum (Levl.) Hutch. 2000g

Cuscuta chinensis Lam. 1000g

Tripterygium hypoglaucum (Levl.) Hutch. ~~Were~~was cut into pieces, extracted three times ~~after~~with 13, 10, 10-~~fold added in~~volume water, each time lasting 1 hour; *Cuscuta chinensis* Lam. was crushed to ~~raw~~coarse powder, immersed in 31-~~fold~~volume water of 80℃ for 1 hour; decoction fluid or immersion fluid of the herbs were filtrated ~~repectively~~respectively, poured across the macropore polymeric adsorbent column, eluted with 70% ethanol, when the color of effluent became deep significantly, collection of the eluent ~~was commenced to collect~~began; when the color of effluent became very weak, elution was over. ~~Eluent of each herbs was recycled to get ethanol,~~The alcohol in the eluent of each herb was recovered. Then the fluid without alcohol was concentrated, dried~~,~~ to obtain the final finally ~~extractive drug~~extract powder ~~was obtained~~; officinal starch was blended with extractive drug powder, and mixed up uniformly, and loaded to 1000 capsules. ~~Dose~~The dose of capsules administered every day, which was prepared with the inventive method thereof, was equivalent to ~~dose of~~ 30g~~/day~~ of crude drugs.

~~Practice example 6:~~

Example of use 6:

Tripterygium hypoglaucum (Levl.) Hutch.2000g

Lycium barbarum L. 1000g

Tripterygium hypoglaucum (Levl.) Hutch. ~~were~~was cut into pieces,

extracted three times ~~after~~with 13, 10, 10-~~fold added in~~volume water , each time lasting 1 hour; *Lycium barbarum* L. was crushed to ~~raw material~~coarse powder, and immersed in 20-~~fold~~volume water of 80℃ for 1 hour; decoction fluid or immersion fluid of the herbs were filtrated ~~repectively~~respectively, poured across macropore polymeric adsorbent column, eluted with 70% ethanol, when the color of effluent became deep significantly, collection of the eluent ~~was commenced to collect~~began; when the color of effluent became very weak, elution was over. ~~Eluent of each herbs was recycled to get ethanol, concentrated, dried, finally extractive drug powder was obtained~~The alcohol in the eluent of each herb was recovered. Then the fluid without alcohol was concentrated, dried to obtain the final extract powder; officinal starch was blended with extractive drug powder, and mixed up uniformly, and loaded to 1000 capsules. ~~Dose~~The dose of capsules administered every day, which was prepared with the inventive method thereof, was equivalent to ~~dose of~~ 30g~~/day~~ of crude drugs.

~~Claims~~

~~1. A pharmaceutical composition for treating rheumatism, characterized in that, it is made from the following materials:~~

~~Tripterygium hypoglaucum (Levl.) Hutch.~~

~~Epimedium brevicornum Maxim.~~

~~Lycium barbarum L.~~

~~Cuscuta chinensis Lam., Cuscuta australis R. Br.~~

~~Wherein the materials must be composed of Tripterygium hypoglaucum (Levl.) Hutch and one or two or three other herbs in the rest 3 herbs.~~

~~2. The pharmaceutical composition according to claim 1 made from the following materials:~~

~~Tripterygium hypoglaucum (Levl.) Hutch. 1-4 part by weight~~

~~Epimedium brevicornum Maxim. 1-4 part by weight~~

~~Lycium barbarum L. 1-4 part by weight~~

~~Cuscuta chinensis Lam., Cuscuta australis R. Br. 1-4 part by weight.~~

~~3. The pharmaceutical composition according to claim 1 made from the following materials:~~

~~Tripterygium hypoglaucum (Levl.) Hutch. 2 part by weight~~

~~Epimedium brevicornum Maxim. 2 part by weight~~

~~Lycium barbarum L. 1 part by weight~~

~~Cuscuta chinensis Lam., Cuscuta australis R. Br. 1 part by weight~~

~~4. The pharmaceutical composition according to claim 1, characterized in that, it can be made from the correspond effective constituents of the~~

~~materials above-mentioned as following that Epimedium brevicornum Maxim.can be replaced by any one or more than one among icariine, deuteron-icariine I, deuteron-icariine II and glyc-icariine A; Tripterygium hypoglaucum (Levl.) Hutch can be replaced by diterpenoids, triterpenoids and alkaloids compound thereof, and Lycium barbarum L.and Cuscuta chinensis Lam.can be replaed by flavone contained thereof.~~

~~5. A method of preparing the pharmaceutical composition according to claim 1, 2 or 3, characterized in that, it includes the processes under-mentioned:~~

~~The raw herbs are weighed, and Epimedium brevicornum Maxim.and Tripterygium hypoglaucum (Levl.) Hutch.were cut into pieces respectively; including raw material or crushed powder of Lycium barbarum L. and Cuscuta chinensis Lam., four herbs hereinbefore, were extracted with 0-95% ethanol at 10-98℃ respectively or combinatively for continuing 1-4 times. Ethanol was recycled respectively or combinatively in extracted fluid, then extraction was concentrated, dried, crushed, mixed uniformly or proportionally, manufactured to dosage form adopted in clinical work;~~

~~Raw herbs were weighed: Epimedium brevicornum Maxim.and Tripterygium hypoglaucum (Levl.) Hutch.were cut into pieces, boiled out in water for three times respectively, and Lycium barbarum L. or Cuscuta chinensis Lam.were immersed in water of 80℃~95℃ for 1-3 times respectively. Decoction or immersion fluids of three times of each herb were blended respectively, then mixture fluid was respectively poured through corresponding macropore polymeric adsorbent column. After absorption, resin column was washed with water until effluent became clear, then was eluted with 30-99.5% ethanol until color of~~

~~effluent became deep. Then eluent was collected until color of eluent became from deep to very weak while ethanol liquid was forced out from the column with water. Eluent was mixed with the ethanol liquid. The weight of total eluent was 1-8 fold of the herbs; eluent of each herbs was recycled, concentrated to specific gavity of 1.10 respectively, then extractive of every herbs were obtained by respective or combinative spray drying, which were mixed uniformly and proportionally, manufactured to dosage form adopted in clinical work.~~

~~6. A method of preparing the pharmaceutical composition according to claim 1, 2 or 3, characterized in that, it can be made into any dose forms adopted in the clinical work such as hard gelatin capsule, soft capsule, tablet, granule and injection.~~

~~7. A method of preparing the pharmaceutical composition according to claim 1, 2 or 3, characterized in that, it includes the processes under-mentioned:~~

~~Tripterygium hypoglaucum (Levl.) Hutch. were cut into pieces, extracted three times after 13, 10, 10-fold added in respectively, each time lasting 1 hour; Epimedium brevicornum Maxim.was cut into segments, extracted three times after 15, 10, 10-fold water was added in respectively, each extraction lasting 1 hour; Lycium barbarum L. was crushed to raw material, and immersed in 20-fold water of 80℃-95℃ for 1 hour; Cuscuta chinensis Lam. was crushed to raw powder, immersed in 31-fold water of 90℃ for 1 hour; decoction fluid or immersion fluid of four herbs were filtrated repectively, poured through WLD or D_{101} or other type of macropore polymeric adsorbent column, eluted with 70% ethanol, when the color of effluent became deep significantly, eluent was commenced to collect; when the color of effluent became very weak, elution was over. Eluent of each herbs was~~

~~recycled to get ethanol, concentrated, dried, finally extractive drug powder was obtained; which were mixed uniformly and proportionally, manufactured to dosage form adopted in clinical work.~~

~~8. The use of the pharmaceutical composition according to claim 1, 2 or 3 in the manufacture of a medicament for treating the rheumatoid and rheumatoid arthritis.~~

~~9. The use of the pharmaceutical composition according to claim 1, 2 or 3 in the manufacture of a medicament for treating the systemic lupus erythematosus.~~

~~10. The use of the pharmaceutical composition according to claim 1, 2 or 3 in the manufacture of a medicament for treating the chronic nephritis, crohn's disease and lepra reaction and the other autoimmune disease .~~

~~Abstract~~

~~The invention has brought to light a kind of antirheumatic and its preparation, which was made from Tripterygium hypoglaucum (Levl.) Hutch, Epimedium brevicornum Maxim, Lycium barbarum L, and Cuscuta chinensis Lam. The invented medicine has the merits of prominent effect, mild side reaction and convenient administration.~~

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